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Frederick L. Knowles, *Editor*

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Office Secretary-Treasurer, S. C. State Hospital, S. C.

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This issue of the Journal

is dedicated to

MARK FREDERICK BOYD

Dr. Mark Frederick Boyd

Secretary-Treasurer, 1930–1945

President, 1946

JOURNAL OF THE
NATIONAL MALARIA SOCIETY

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NATIONAL MALARIA SOCIETY PRESENTS SILVER SERVICE TO DR. MARK F. BOYD

High-lighting the proceedings of the thirty-first annual meeting of the National Malaria Society, in Miami, Florida at the Hotel Everglades, Nov. 4–7, 1946, was the presentation of a silver service to Dr. Mark F. Boyd, the retiring president. Remarks by Dr. Louis L. Williams, Jr., who made the presentation on behalf of the Society, are as follows:

Members of the National Malaria Society:

As our President Dr. Mark Boyd, retires from his office at this meeting thus closing a period of nearly twenty years of active service as an officer of the Society, we wish to express to him our appreciation for the great services he has rendered the Society and to present to him a token of our appreciation and affection.

The Society commenced in 1916 as the National Malaria Committee organized by Dr. Frederick Hoffman. It was commenced primarily with the view of bringing together the few students of malaria in the United States in the hope that through review of the therapeutic field a standard treatment of malaria might be promulgated. During World War I the meetings of the Committee proved such a good medium of exchange of information and was so valuable to the malaria control workers during the war period that arrangements were made to meet annually and it became in effect the Malaria Section of the Southern Medical Association. At first its transactions were published by the United States Public Health Service and subsequently the most important papers were published by the Association's Journal. The meeting place was provided by the Southern Medical Association but it soon appeared that the Committee could not continue as a separate section in light of the development by the Association of its Section on Public Health. At that time we constituted ourselves a separate society but continued to meet with the Southern Medical Association and continued the privilege of publishing more important papers in the Association's Journal. Space limitations precluded publication of so many papers and the mimeograph proved an inadequate substitute. Dr. Boyd's development of the Society solved our difficulties and it is to him you wish me to express our thanks.

This is the pleasantest task to which the Society has ever assigned me, the task of telling a distinguished member what we think of him. I had thought that for once I would be speaking to this audience on other than a scientific subject. On reflection however, it *is* obvious that the subject is a scientific one so I am going to address *him* directly.

Mark, I have talked to so many of your colleagues and read so many of the letters, which have come in from all parts of the country and from over-seas, ever since it

Photo by Marvin F. Carter
Memphis & Shelby Co. Health Dept.

Part of the group which attended the afternoon sessions of National Malaria Society on Nov. 5, 1946; taken on the terrace of the Hotel Everglades, Miami, Fla.

was known that there would be an opportunity to express their appreciation of your services, that I am sure I speak for all of us.

We have know you as an author on public health, on malariology and as an investigator of malaria problems. As a young man you commenced the study of mosquito larval foods and environments. These studies finally opened the way to a particular classification of ponds. You were the first to establish insectaries for breeding anophelines. Insectaries have become essential in solving many malaria problems and were particularly valuable in war time in developing the newer therapeutic agents. Your work in strain immunity cleared up confused thinking in the immunology of malaria. Mark, I could go on and on and yet not cover your valuable contributions to the epidemiology, parasitology and therapy of malaria; your studies of the entomology and eccology of anophelines; or your work in the control of malaria. We all know your considerable labors in building up the National Malaria Committee from its position as the Malaria Section of the Southern Medical Association dependent upon it for limited publication, into the full fledged society it is today with its own Journal, and this we like. More than this we like the feeling you have engendered by the great and kindly interest you have taken in the work of each of us. We think that perhaps you do not know and we want you to know that one of your greatest works has been this stimulation to malaria control workers, a stimulation exceeded by none and equalled surely by no other except LePrince.

These are some of the reasons Mark, why the National Malaria Society wishes to present to you this silver service as a token of appreciation on the occasion of your leaving office after serving in various capacities for so long.

This is the inscription:

"Presented to Dr. Mark Frederick Boyd in recognition of his long and distinguished service to the Society and of his contributions to the knowledge of malaria and its control, by the National Malaria Society, November 7, 1946."

Whenever you notice this gift through the years we hope you will regard it as a symbol of our appreciation of your scientific achievements and of the admiration and affection of your colleagues in the National Malaria Society.

BIOGRAPHICAL DATA AND BIBLIOGRAPHY OF MARK FREDERICK BOYD

I. Born: St. Paul, Minnesota, U. S. A., May 21, 1889. Son of William Samuel and Maria (Moench) Boyd.

II. Residence: 615 East Sixth Avenue, Tallahassee, Florida.
Address: P. O. Box 997, Tallahassee, Florida.

III. Professional Education: M.D., State University of Iowa, 1911. M.S., (Med) University of Iowa, 1913. C.P.H., Harvard University and Massachusetts Institute of Technology, 1914.

IV. Professional Career:

(a) Student Assistant in Pathology and Bacteriology, University of Iowa, 1910–11.

(b) Instructor Pathology and Bacteriology, University of Iowa, 1911–12.

(c) Health Officer, Oskaloosa, Iowa, 1912–13.

(d) Charles Follen Folsom Teaching Fellow in Hygiene, Harvard Medical School, 1913–14.

(e) Associate Professor Bacteriology and Hygiene, University of Nevada, 1914–15.

(f) Associate Professor Preventive Medicine, Unkversity of Iowa, 1915–17.
Epidemiologist, Iowa State Board of Health, 1915–17.

(g) Professor Bacteriology and Preventive Medicine, Medical Department, University of Texas, 1917–21.
Visiting Physician John Sealy Hospital, Galveston, Texas, 1917–21.

(h) Member Field Staff, International Health Division, Rockefeller Foundation, 1921 to 1946. Director: Field Studies of Malaria in Brazil, 1922–23; of Malaria Field Study Stations in Leesburg, Ga., 1925; Edenton, N. C., 1926–1928; Jamaica, B.W.I., 1928; Division Malaria Control, Mississippi State Board of Health, Jackson, 1929–30; Station for Malaria Research (Florida State Board of Health), Tallahassee, 1931–1946.

V. Honorary Appointments:

(a) Member Nevada State Board of Health, 1914–15.

(b) P.A. Surgeon (Reserve) U. S. Public Health Service, 1920–22, in-charge U. S. P. H. S. Plague Laboratory, Galveston, Texas, 1920.

(c) Formerly corresponding and later expert member, Malaria Commission, Health Section, League of Nations.

(d) Former Member Board of Malaria Consultants, Tennessee Valley Authority.

(e) Delegate U. S. Government to 3rd International Congress Tropical Medicine and Malaria, Amsterdam, 1938.

(f) Delegate U. S. Government to 10th American Sanitary Congress, Bogota, 1938.

(g) Advisor, Malaria Committee, Pan American Sanitary Bureau.

(h) Member Board of Scientific Advisors, Gorgas Memorial Institute.

(i) Visiting Physician, Florida State Hospital.

(j) Member Board of Directors, Gorgas Memorial Institute (1945), *ex officio* as President American Academy of Tropical Medicine.

(k) Member Sub-Committee on Tropical Diseases, Division Medical Sciences, National Research Council.

(l) Member Commission Tropical Diseases of Board for Investigation Epidemic Diseases in U. S. Army (Consultant Secretary of War).

(m) Civilian Consultant to Secretary of War, in Medicine Division, Surgeon General's Office.

(n) Lecturer Army Medical School (Consultant Secretary of War).

(o) Editor, American Journal of Tropical Medicine (1947).

VI. Membership in Scientific Societies:

(a) Member National Malaria Society (Secretary-Treasurer 1930–1945; President, 1946).

(b) Member American Academy of Tropical Medicine (President, 1945).

(c) Member American Society of Tropical Medicine (President, 1938).

(d) Honorary Member Sociedad Mexicana de Medicina Tropical.

(e) Corresponding Member Societe Belge de Medecine Tropicale.
(f) Fellow Royal Society of Tropical Medicine.
(g) Fellow American Public Health Association.
(h) Fellow American Association Advancement of Science.
(i) Fellow Iowa (emeritus) and Florida Academies of Science.
(j) Corresponding Member Academia Nacional de Medicina de Mexico.

VII. Bibliography on Medical and Public Health Subjects.

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92. On the infectiousness of patients infected with *Plasmodium vivax* and *Plasmodium falciparum* (with KITCHEN, S. F.). Am. Jour. Trop. Med., XVII (1937), 253–262.
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A REVIEW OF STUDIES ON IMMUNITY TO VIVAX MALARIA[1]

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Having been exclusively occupied during the past quarter of a century in the study of malaria, and having during a considerable portion of this time enjoyed unique opportunities for work with induced infections, it is natural, if not inevitable, that I should select for this occasion a theme which is related to this topic. Since the question of immunity to malaria infections has continuously held my interest during this period, it may be useful to review the isolated contributions which my co-workers and I have made to the subject, and with the perspective of time to fit them into a comprehensive picture at a definite focal point.

Induced malaria infections are widely employed in the therapy of neurosyphilis. Most commonly, inoculations to produce such infections are made with parasites in the trophozoite stage contained in infected blood; less frequently the inoculations are done with sporozoites and effected by the application of infected mosquitoes to the patient. Since very few autochthonous infections originate from trophozoites, the former method may be distinguished as artificial inoculation, while the latter may be termed natural inoculation, since it induces infection in the manner in which most autochthonous infections are initiated. The course and character of the infections induced by either method of inoculation are indistinguishable, although it may be said that those artificially induced are less chronic and are readily eradicated by adequate plasmodicidal therapy, while those naturally induced endure longer, manifest clinical exacerbations after protracted remissions and are difficult to eradicate by specific therapy.

The outcome of the inoculation of an institutionalized neurosyphilitic patient usually cannot be predicted. This uncertainty is not attributable to the possible absence of parasites from the inoculum, as their presence is readily verifiable, but to the circumstance that the mental deterioration of a patient of this type is such that he is rarely able to give an adequate or accurate history of any prior experience with malaria. Frequently the only lead is afforded by knowledge of the patient's birthplace, which, if outside known endemic territory, justifies a presumption of susceptibility. This presumption in the case of such individuals is confirmed by an invariable take, which is followed by a clinical attack lasting 3 weeks or longer, and a more persistent and slowly subsiding parasitemia (for example, fig. 1). Since we have never seen an attack of such duration follow known homologous or heterol-

[1] The Presidential Address, delivered at the 29th annual meeting of the National Malaria Society at Miami, Fla, November 5–7, 1946. The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in coöperation with the Florida State Board of Health and the Florida State Hospital.

ogous reinoculation, a long attack is considered indicative of previous complete susceptibility.

Other patients may or may not experience a take. In those with a take, the numbers of the parasites rise about as rapidly as in the susceptibles, but the maximum attained is at a relatively low density, and is either transitory or sustained for only a few days. Without therapeutic interference, the infection suddenly subsides and disappears. Depending upon the duration of the period at which high parasite densities persist, clinical activity may be evident for from one to approximately 14 days,

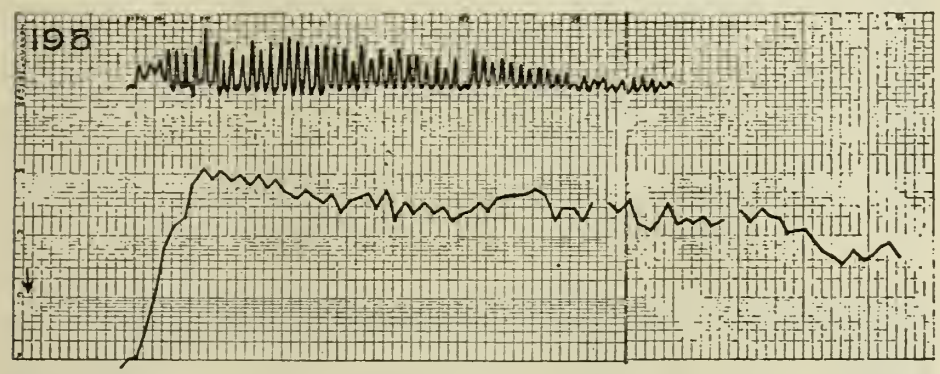

FIG. 1. Naturally induced vivax infection terminating spontaneously. Prepatent period eleven days, incubation twelve days, first clinical reaction with density of 10 parasites per cubic millimeter. Remittent fever from 12th to 14th day, insensibly changing to intermittent quotidian. Spontaneous suppression of the paroxysm on the 47th day unexplained. Note the gradual decline in temperature from maximum of 107°F in paroxysm on 19th day to the 59th day when it did not attain 100°F. Maximum parasite density of about 12,000 per cubic millimeter also on the 19th day. Clinical activity ceases spontaneously with concurrent parasite density of about 1,800 per cubic millimeter and is still in excess of 400 per cubic millimeter on 94th day from inoculation when enumeration was discontinued.

This and the following charts represent the day by day progress of (a) the clinical activity of the infection as reflected in the temperature and (b) the parasite density. The first is displayed in the upper portion of the chart and represents the temperature curve in degrees Fahrenheit taken at four-hour intervals. The lower portion, on a horizontal logarithmic scale, represents by a solid line the density of the total parasites (trophozoites and gametocytes) per cubic millimeter as determined from smears routinely taken about 8 a.m. The lowest line of the first cycle of horizontal ruling represents a density of 10 parasites per cubic millimeter, and the corresponding line in subsequent cycles 100, 1000, 10,000, and 100,000 parasites. The vertical lines mark the days elapsing since inoculation by means of infected mosquitoes, the day of which is further marked by the arrow.

terminating abruptly coincident with a sudden fall in parasitemia (fig. 2, chart 8); (fig. 3, charts 9, 10). Less frequently observed are the patients in whom the parasitemia rises to a low peak, from which it rapidly falls without producing any clinical activity (fig. 2, chart 6). In rare instances there is encountered a patient in whom no take results despite inoculation with a proven inoculum. The status of the patients in the last three categories may be inferred from the course of events following the reinoculation of patients who were originally, judging from the character of their primary infection, completely susceptible.

In figure 2 (5) there is presented a chart showing the experience of a patient

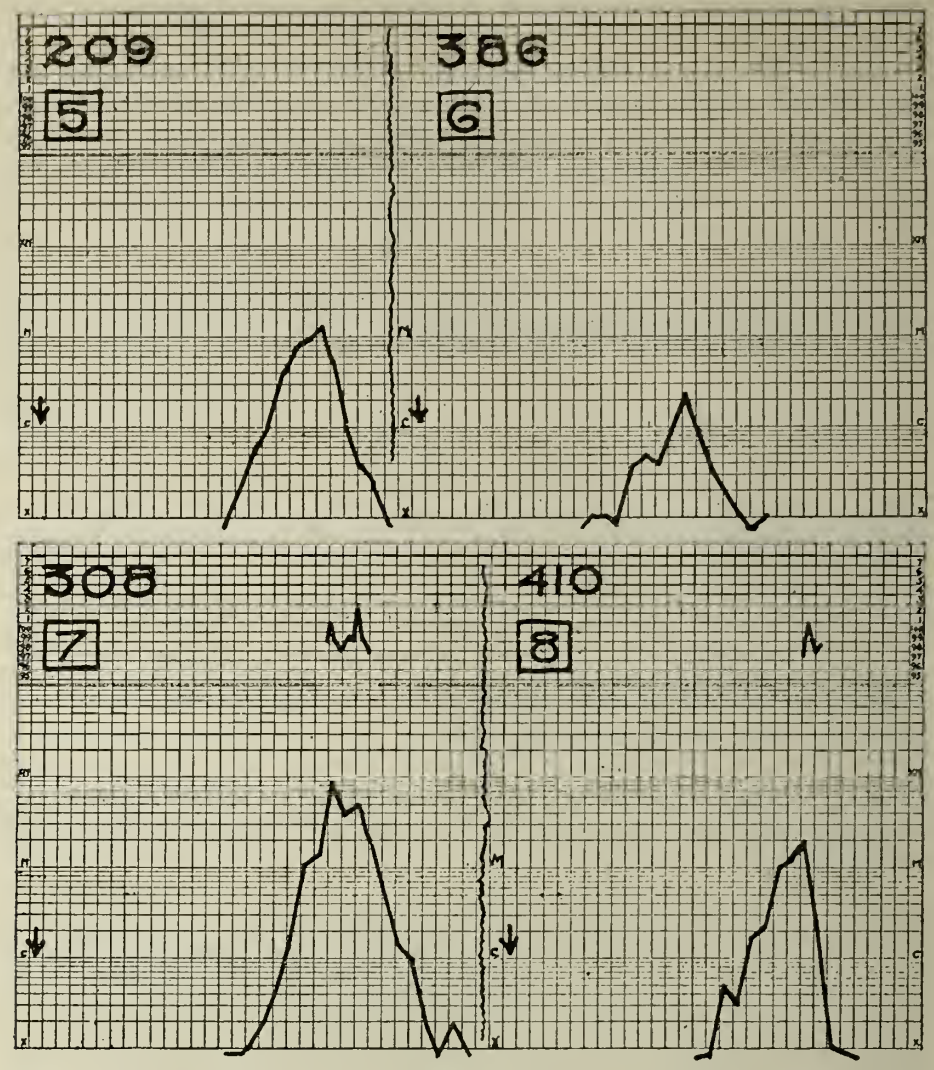

FIG. 2

Chart 5. Patient 209. Results of known homologous reinoculation. Short subclinical parasitemia. Note the low density of parasites even at the maximum, and rapid decline.

Chart 6. Patient 386. Results of primary inoculation on service with the McCoy strain. A short subclinical parasitemia, identical with previous chart.

Chart 7. Patient 308. Results of known heterologous reinoculation. Primary inoculation with the McCoy strain (not shown), reinoculation with the Ohio strain. Brief parasitemia, with three-day period of clinical activity. Note similarity of parasite curve with that seen in the other charts. In all the decline is rapid.

Chart 8. Patient 410. Results of primary inoculation on service with the McCoy strain. Brief parasitemia with clinical activity for one day only.

subsequent to reinoculation with the same (homologous) strain of parasite which produced his original infection. The parasitemia is transitory, without any clinical activity at the time of its maximum. On the other hand, the patient whose experience is shown in figure 2 (7), was reinoculated with a different (heterologous) strain of parasite from that which produced the original attack. A 3-day period of clinical activity coincides with the maximum parasite density. In both instances the parasitemia declined to submicroscopical levels as rapidly as it rose. It appears justifiable therefore to conclude that patients who, after their initial therapeutic inoculation, presented infections similar to those shown in charts 6, 8, 9 and 10 (figs. 2 and 3), were not pristinely susceptible at the time of inoculation, but had had previous experience with malaria.

It is further concluded from the similarity between charts 5 and 6 (fig. 2) that the experience of the patient shown in chart 6 must have been with the identical strain of parasite we employed, while comparison of chart 7 (fig. 2) with charts 8, 9, and 10 (figs. 2 and 3) leads to the conclusion that the earlier experience of the latter patients was with a strain or strains different to that employed in the induced inoculations (Boyd, 1942).

It may be alleged that the results represented by charts 5 and 6, and also the outright failures, are attributable to either a deficiency or entire lack of parasites in the inoculum. While not denying that at times the outright failures may be due to this cause, such instances usually can be excluded when the mosquitoes employed are dissected and the presence of sporozoites is verified. Even if present in the mosquitoes, the sporozoites might be deficient in numbers or vitality. In the latter event they may be expected to show the fishhook or horseshoe appearance, indicative of degeneration, which they invariably undergo if the infected mosquitoes are preserved a sufficient length of time (Boyd and Stratman-Thomas, 1934). This is progressive, and will be complete after 50 days' storage of the mosquitoes at about 4°C. Consequently in order to demonstrate that patients who were originally susceptible have, with recovery from their infection, acquired an immunity to the strain of parasites which produced the attack, it must be shown that their reinoculation was effected with an adequate infecting dose of parasites. This may be accomplished by applying the mosquitoes used in the inoculation of the test patient, previously and subsequently to susceptible control patients. If the control patients acquire an infection, it is certain that the reinoculated patient received an infecting dose of sporozoites (Boyd and Stratman-Thomas, 1933).

In figure 4 there is shown the result of homologous reinoculation of a test patient, whose primary attack with the McCoy strain lasted 42 days. At the time of reinoculation with the McCoy strain 63 days later, parasites of the first infection were readily demonstrable. The prior control was inoculated on December 3 and 4 by a total of 4 mosquitoes. The same mosquitoes were applied to the test patient on December 6, and to the post control on December 8 and 9. All 4 mosquitoes fed once on each patient, and on final dissection all were found to have sporozoites in their salivary glands. The controls developed verified clinical malaria after incubation periods of 14 and 13 days, while the test patient did not exhibit any clinical reaction.

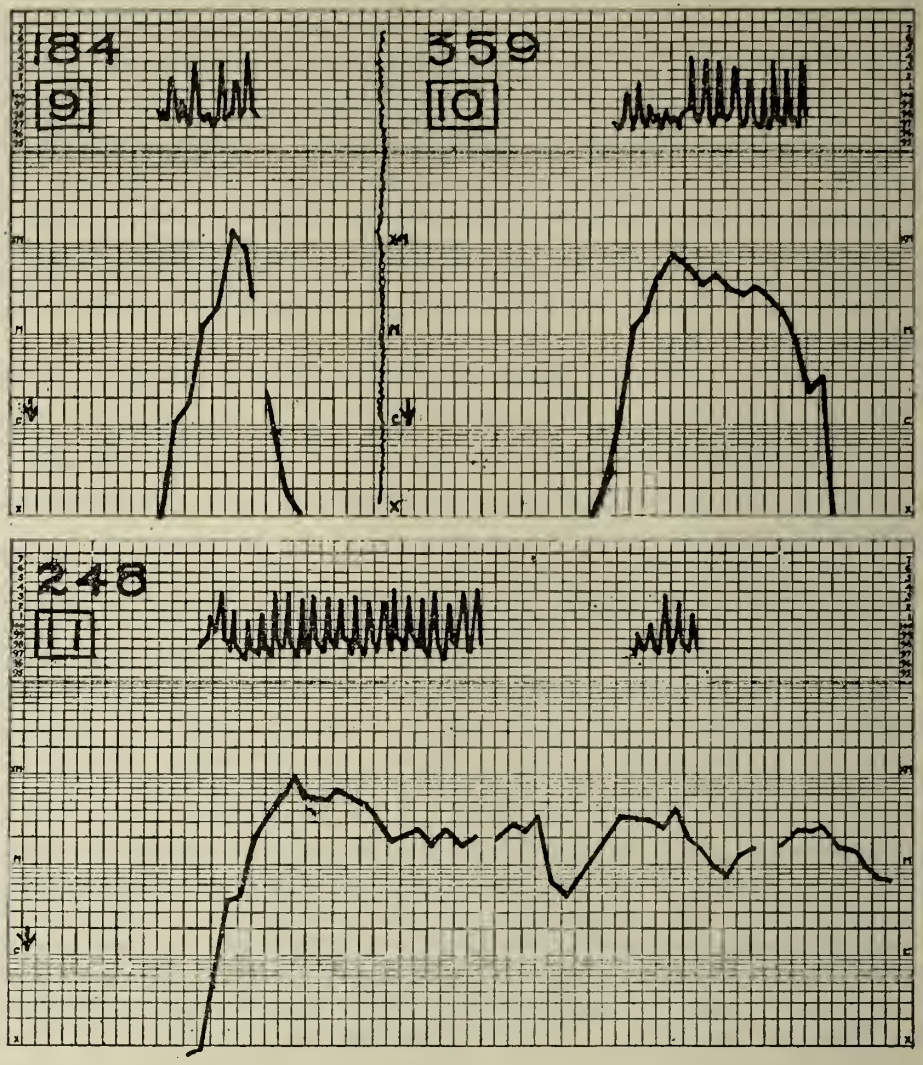

FIG. 3

Chart 9. Patient 184. Results of primary inoculation on service with McCoy strain. Brief rapidly subsiding parasitemia, with clinical activity for seven days.

Chart 10. Patient 359. Result of primary inoculation on service with McCoy strain. Duration of parasitemia longer, but subsidence nevertheless is rapid; clinical attack lasted 14 days.

Chart 11. Patient 248. Result of primary inoculation on service with McCoy strain. Parasitemia gradually declines. Continuous clinical activity for three weeks, remission of 11 days, followed by a recrudescence of five days (*cf.* Fig. 1).

The reinoculation in another similarly executed experiment was with a heterologous strain (fig. 5). The test patient had a primary attack with the Cabler strain, which lasted until its interruption, 25 days later. At the time of reinoculation with the

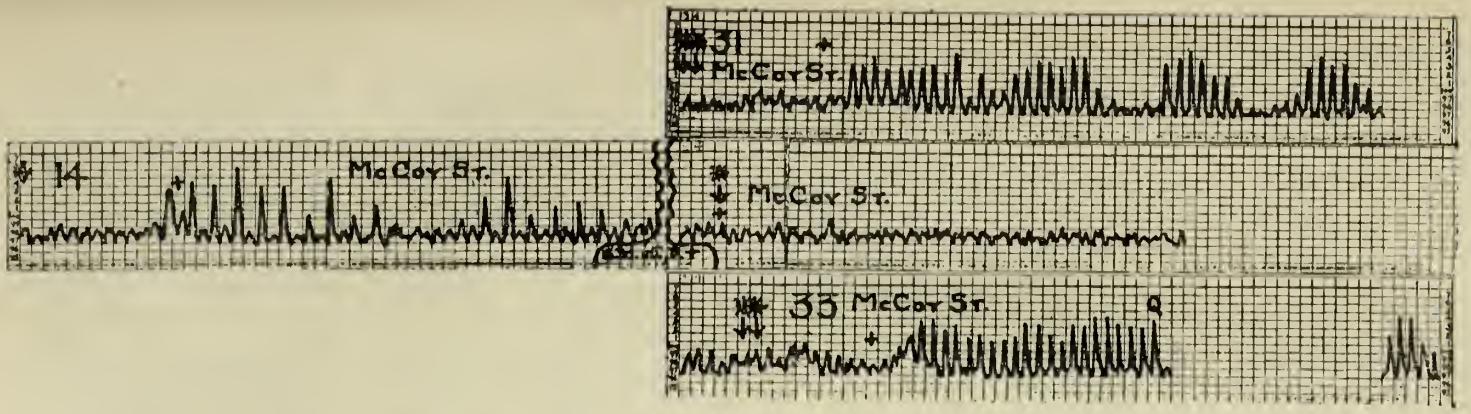

FIG. 4. Homologous reinoculation of a convalescent from infection with the McCoy strain (Patient 14), the course of the primary infection being shown in the left part of the chart. The mosquitoes used in the reinoculation were previously applied to patient 31 and subsequently to patient 33. Both of these controls developed clinically active infections, but the test patient did not react.

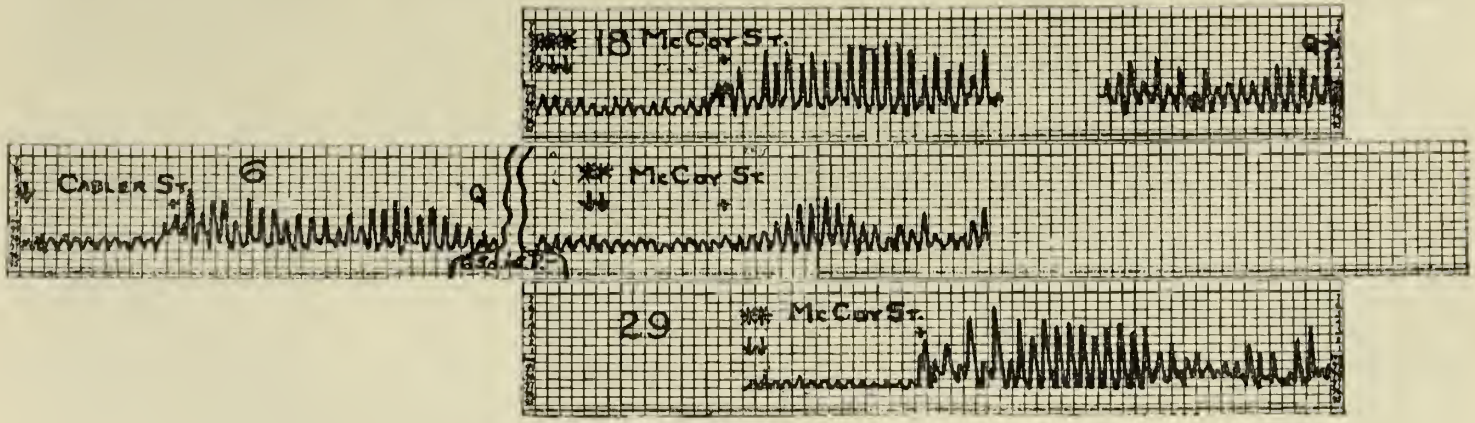

FIG. 5. Heterologous reinoculation of a convalescent from infection with the Cabler strain (Patient 6), the course of which is shown to the left, by the McCoy strain. The mosquitoes used in the reinoculation were previously applied to patient 18 and subsequently to patient 29. Both of the controls as well as the test patient developed clinically active infections.

McCoy strain, his smears had become negative subsequent to the administration of 30 grains of quinine. On October 7, 8 and 9, the prior control was inoculated once by a total of 4 mosquitoes. Three of these fed on the test patient on October 11 and 12, and 2 on the post control on October 24 and 25. Sporozoites were only found in the glands of the 2 mosquitoes which survived to bite all 3 patients. The prior and post controls developed verified malaria after incubations of 14 and 14 days, while the test patient, after an incubation of 14 days, developed an attack which lasted 13 days, and after a 2-day remission, presented a 2-day recrudescence.

The minimal infecting dose of parasites is, in so far as it relates to susceptible patients, apparently very small. It has been ascertained (Boyd and Kitchen, 1943) by careful dilution of infected blood that infections can reliably develop following intravenous introduction of as few as 10 trophozoites (incubations of 12 to 15 days), and a take has been secured following inoculation of a presumably single trophozoite isolated in a micromanipulator. We lack comparable data for sporozoites, although believing they would exhibit similar potency. Ordinary routine inoculations are commonly effected with a great surplus of sporozoites, provided by several infected mosquitoes, although a take may result from the mere insertion of the proboscis of a single infected mosquito. It has been shown further (Boyd, 1940) that, considering dosage in the crude terms of the number of infected mosquitoes applied, the duration of the incubation period within certain limits varies inversely with the dose of sporozoites, while the duration of the clinical attack tends to vary directly therewith.

Referring back to figure 1, representing the course of a vivax infection in a person who originally was completely susceptible, it will be noted (1) that clinical activity was initiated by very low parasite densities, not exceeding 10 per c.mm. of blood; (2) that for about a week after the infection developed, there was a steady and progressive increase in the parasite density until the attainment of the maximum, when between 10,000 and 20,000 parasites per c.mm. were observed; (3) from this point the general trend was gradually downward until the cessation of clinical activity some 8 weeks later, when a density of about 2,000 parasites per c.mm. prevailed; (4) thereafter and as long as observations continued over a period of about 4 weeks the rate of parasite decline was slightly accelerated, but they nevertheless continued to be fairly abundant. It is apparent that experience with a malaria infection brings about certain changes in an originally susceptible patient (Boyd, 1944). The low density of parasites at the onset is in marked contrast to that prevailing at the end of the active infection, which suggests that the patient has been acquiring a tolerance to them. The rapid climb in parasite density during the week following their first appearance is an expression of exuberant growth, during which interval the parasites enjoy the maximum advantage of their multiplication potential. This surge is soon checked, and the subsequent trend in density shows that at later periods of division the effective multiplication progressively declines. Some agency is now operating to destroy most of the parasite progeny. In the case of the reinoculated patients (fig. 2, charts 5 and 7), it will be noted that the rate of increase in the inital surge is about the same as in the susceptible, but it is brusquely checked at a lower maximum, and that the parasite numbers diminish thereafter as rapidly as they rose. Furthermore, it will be seen that these patients did not experience clinical activity

until much higher parasite densities were attained. It appears that, as a consequence of their earlier malaria experience, the first infection sensitized some mechanism of the body to the parasites to such a degree that it was quickly activated following the reinoculation, thereupon expeditiously removing the new invaders. Although checking of the multiplication potential of the parasites and the acquirement of a tolerance to the presence of the residue are early manifest, ability to remove rapidly those subsequently introduced develops much more slowly, and during the period of latency.

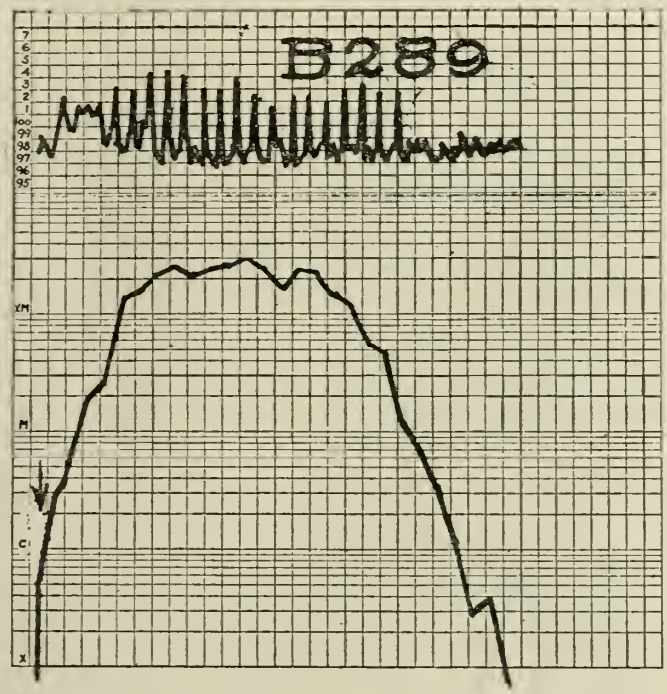

FIG. 6. Patient B-829, intravenously inoculated with blood containing 180 million parasites of the McCoy strain on 6/1/35. Note that parasites were immediately detectable thereafter, and that clinical activity, initiated on the following day, must be attributed to the parasites of the inoculum. There has been suppression of the prepatent and incubation periods. Control to patient shown in following chart.

This defense mechanism, particularly when confronted with homologous parasites, is capable of expeditiously dealing with excessively heavy doses of trophozoites. It has been shown that intravenous inoculation with 19 million or more trophozoites will permit the immediate microscopical detection of the parasites of the inoculum in smears from the recipient, and that clinical activity, due to the multiplication of the parasites of this alone, will initiate clinical activity within 48 hours (fig. 6) (Boyd and Kitchen, 1936b). Yet similar quantities of the homologous parasites can be introduced into recent convalescents whose primary parasitemia is still patent and, although they will thereafter show a slight resurgence in their parasitemia, there is no

clinical reaction. This superinfection will reach a crest in a week or 10 days. If their primary infection is less recent and no longer patent, the superinfection may be patent for a week or 10 days. This immunity remains potent for several years, even in the apparent absence of a latent infection. Figure 7 shows the homologous reinoculation of one patient 3½ years after his primary infection. A patient who was inoculated with 10 cc. of the blood of the former, removed prior to the reinoculation, did not develop an infection, which makes the current persistence of a latent chronic infection from the first inoculation appear unlikely. This patient was again homologously reinoculated after the lapse of a further interval of 3½ years, or a total of 7

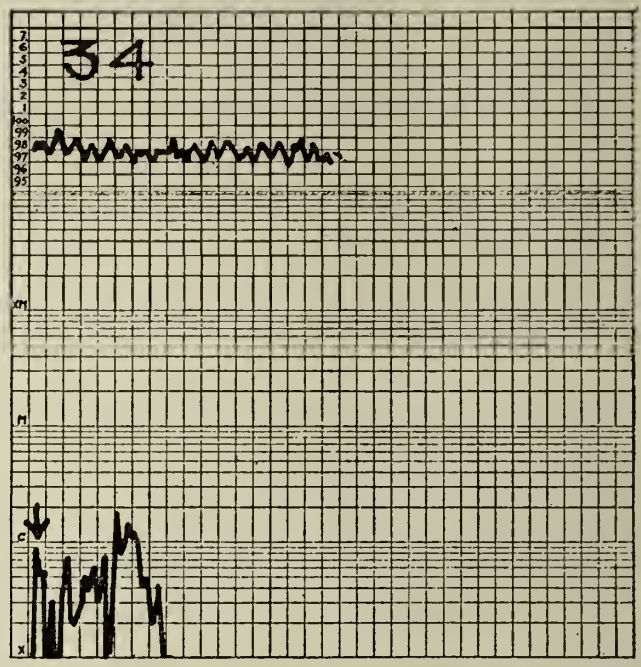

FIG. 7. Patient 34, homologously reinoculated on 6/1/35, 3½ years after primary infection with the McCoy strain, with 180 million trophozoites injected intravenously. The subclinical parasitemia persisted about a week.

years from his primary infection, without experiencing more than a transient parasitemia from the reinoculation. Acquired immunity is thus seen to be not only very potent, but to have appreciable endurance.

On the other hand, the development of homologous immunity to two strains of parasites in convalescents who had been simultaneously inoculated with two strains in approximately equal numbers, appears weaker than that exhibited by patients infected with but a single strain, as reinoculation with either strain is commonly followed by further clinical activity (Boyd, Kupper and Mathews, 1938).

It will be recalled that reinoculation of a convalescent with the homologous strain, in the instances presented, resulted in the patient's exhibiting a subclinical parasitemia,

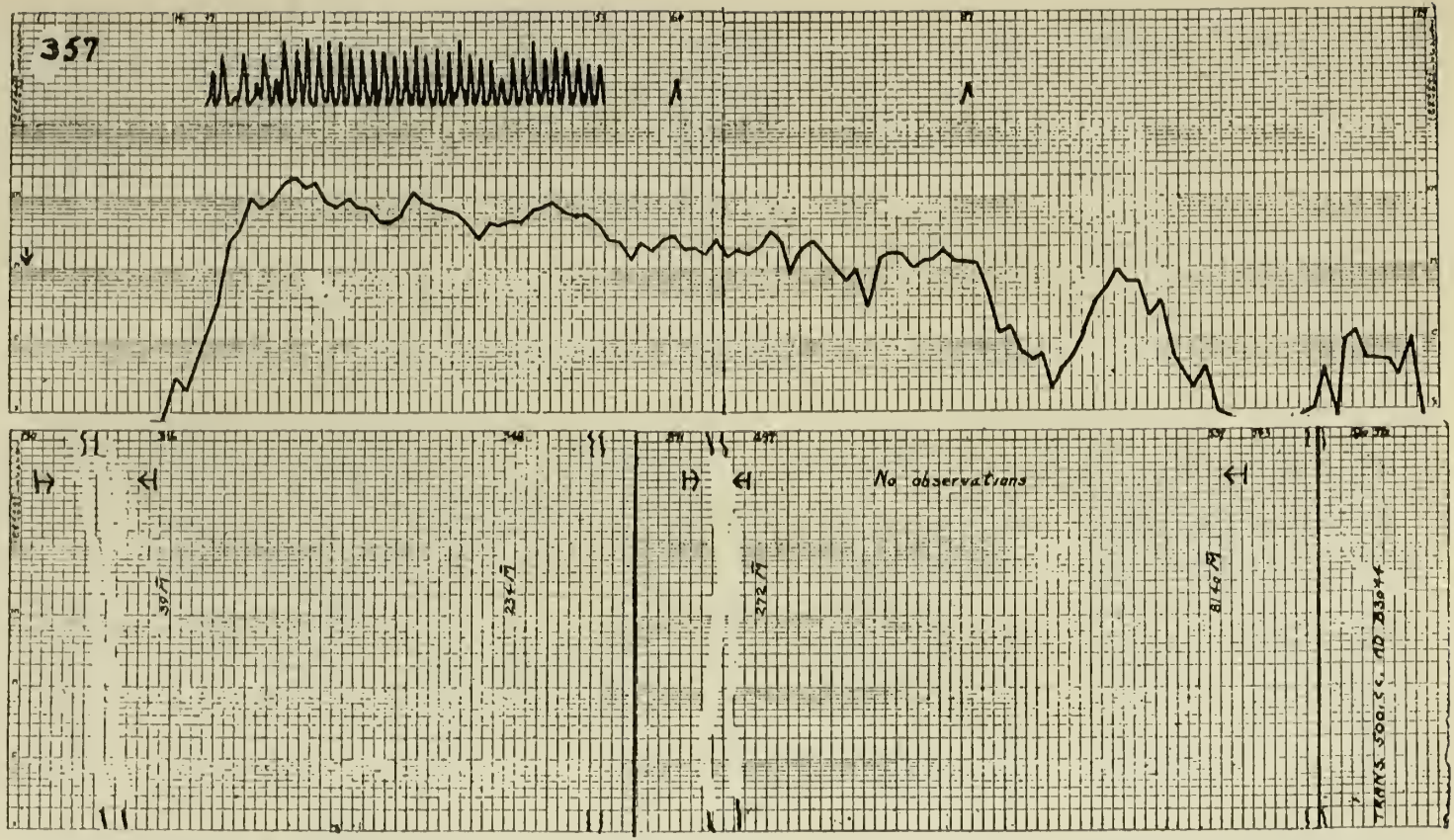

FIG. 8. Patient 357. Showing administration of three hyperimmunizing doses (middle) of homologous parasites to a patient originally fully susceptible to the McCoy strain (top), without subsequent parasitemia or clinical activity, neither of which became apparent following a further inoculation with over 8 billion parasites. The bottom portion shows when the patient served as the donor.

with characteristics suggestive of a superinfection. This logically raises the question as to whether a series of reinoculations with the homologous strain might reinforce or exaggerate this immunity so that the patient would become able to remove large numbers of trophozoites promptly without exhibiting this subclinical parasitemia. If attainable this may be considered a state of hyperimmunity.

In order to explore this possibility, it was decided to utilize convalescents whose protracted primary attack, and more protracted primary parasitemia, indicated that prior to their original inoculation they had been pristinely susceptible. After some preliminary trials it appeared best to defer the first reinoculation until the primary parasitemia had subsided to submicroscopical levels, and to space the reinoculations at not less than monthly intervals (Boyd and Kitchen, 1943). This series of ob-

TABLE 1

Results of Repeated and Simultaneous Homologous Reinoculation of Convalescents from an Infection with P. vivax (McCoy Strain)

PATIENT	ORIG. INFECTION	DURATION OF PRIMARY			TERM. ATTACK	REINOCULATIONS				
		Prepat. period	Incub. period	Attack		No.	Day from first inoc.	Prior parasitemia	Dose millions parasites	Result
		days	*days*	*days*						
357	Nat. ind.	14	17	37	Spont.	(1)	316	Neg.	39	Neg.
						(2)	348	Neg.	234	Neg.
						(3)	497	Neg.	272	Neg.
						(4)	539	Neg.	8,140	Neg.
						(5)	692	Neg.	52	Neg.
375	Nat. ind.	15	14	43	Spont.	(1)	159	Neg.	39	Neg.
						(2)	191	Neg.	234	Neg.
						(3)	340	Neg.	272	Neg.
						(4)	382	Neg.	8,140	Neg.
						(5)	536	Neg.	105	Neg.

servations culminated in the immunization of two patients. The results for both patients are presented in Table 1; those for one are shown graphically in figure 8.

These convalescents already had a very potent immunity when the series of homologous reinoculations was begun, as neither the first nor any subsequent reinoculation resulted in any return of a detectable parasitemia, or, for that matter, in any clinical reaction. Finally, at the fourth reinoculation, each received an enormous injection of over 8 billion of the homologous parasites, with altogether negative results. This inoculation necessitated a transfusion with 100 cc. of infected blood. When it is considered that this is at least 800 million times the minimal infecting dose, it is seen that they possessed a formidable immunity.

Should this immunity have humoral characteristics in any appreciable degree, it is reasonable to expect that it might be passively transmitted, and its action be manifest in infected recipients. Consequently hyperimmune Patient 357 served as a donor of a 500 cc. transfusion to patient B-3044, given practically simultaneously

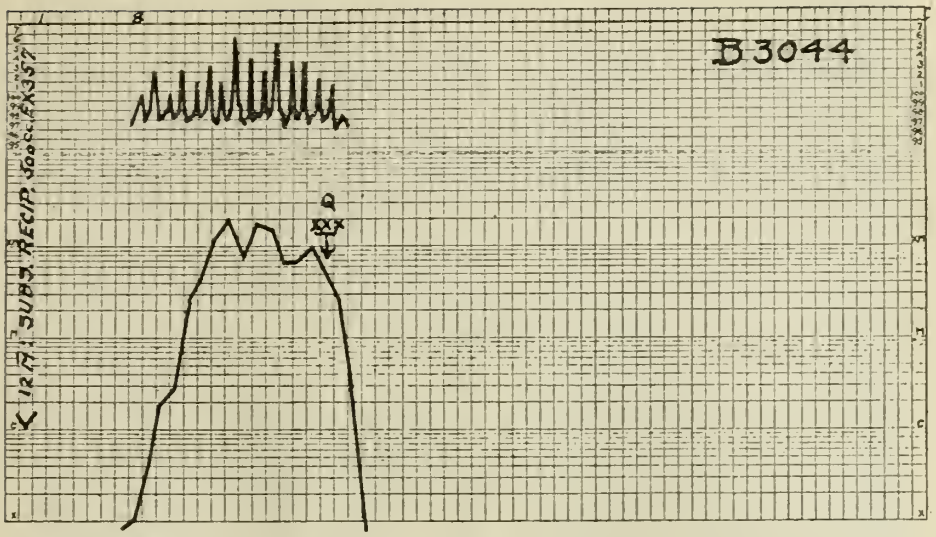

FIG. 9. Patient B-3044. Recipient of a transfusion from patient 357 immediately after an inoculation with 12 million parasites of the McCoy strain. No effect discernible.

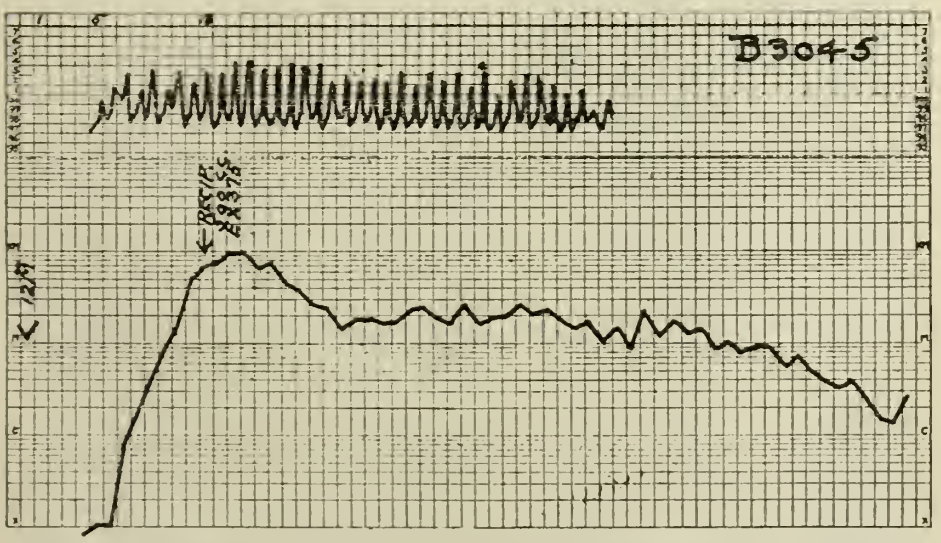

FIG. 10. Patient B-3045. Recipient of a transfusion from patient 375 two weeks after an inoculation with 12 million parasites of the McCoy strain, when the infection was fully developed. No effect discernible.

with the inoculation of the latter with 12 million parasites of the same strain. The results, shown in figure 9, do not suggest that the inoculation was adversely affected by the transfusion. Another patient, B-3045, was similarly inoculated with 12

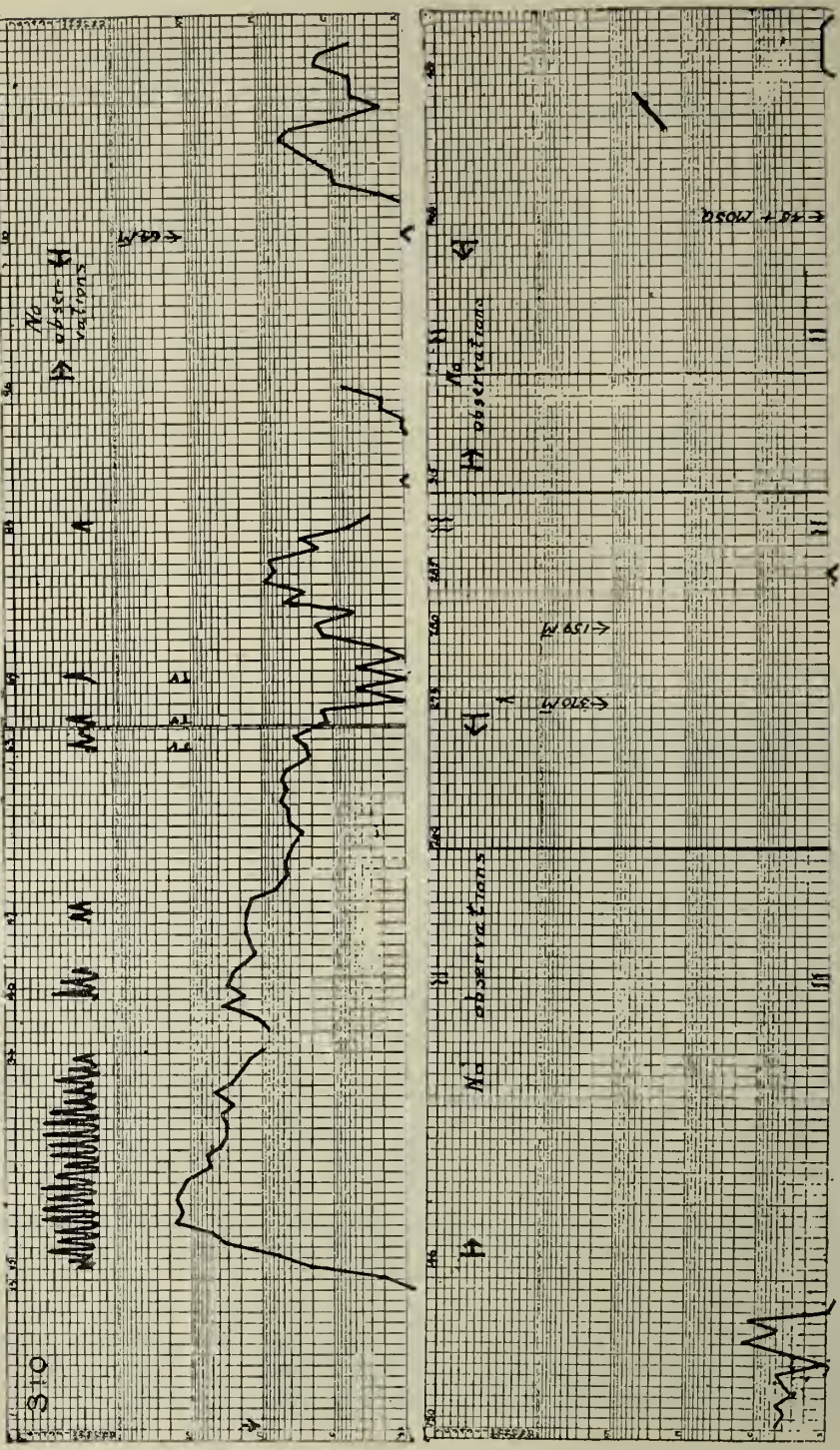

FIG. 11. Patient 310, hyperimmunized to the McCoy strain following convalescence from an attack. Reinoculated by 45 infected mosquitoes (bottom), parasites reappear in about two weeks and remain at minimal densities for five days.

million parasites, and on the 13th day thereafter, when the parasitemia was about 8,000 per c.mm. and the clinical attack was in its 9th day, was the recipient of a 500 cc. transfusion from hyperimmune Patient 375 (fig. 10), without any discernible effect therefrom on either the parasitemia or the clinical course. This suggests that the immunity of the hyperimmunes is cellular rather than humoral in character.

The observations cited indicate that the mechanism of the homologous immunity evident in a vivax convalescent is exerted against the trophozoite stages of the

TABLE 2

Attempt at Active Immunization of Susceptible Patients by Inoculation with Attenuated Sporozoites (P. vivax, McCoy)

PATIENT	MOSQUITO LOT	AGE SPOROZOITES 1ST APPLICATION	APPLICATION ON					
			3/27		4/3		4/10	
			Appl.	Pos.	Appl.	Pos.	Appl.	Pos.
		days						
176	299	55	17	9	17	9	17	9
177	299	55	16	10	16	10	16	10
178	299	69					16	5
179	299	69					16	2

TABLE 3

Test Inoculation of Patients Inoculated with Attenuated Sporozoites

PATIENTS		DATE INOC.	DAYS SINCE LAST APPL. ATTENUATED SPOROZOITES	MOSQUITO LOT	NUMBER INFECTIOUS MOSQUITOES CONSECUTIVELY APPLIED		INCUBATION	ATTACK
Controls	Test				Appl.	Infect.		
180		4/13		308	9	8	8	31
	177	4/17	7		7	7	9	10
	179	4/20	10		6	6	10	4
181		4/24			6	6	13	62
182		4/13		308	9	9	10	11
	176	4/17	7		8	8	9	32
	178	4/20	10		7	7	11	16
183		4/24			7	7	12	15

parasite. It is acquired regardless of whether the primary attack is artifically induced (by trophozoites) or naturally induced (by sporozoites). However it does not appear that this immune mechanism is capable of dealing with subsequently introduced homologous sporozoites, as some at least of these cells survive, to produce, in as yet unknown manner, the trophozoites of the subclinical infection (Boyd and Kitchen, 1936a).

The significance of this is shown in fig. 11, which illustrates an experiment in which a convalescent from a naturally induced infection was reinoculated with 69 million, 370 million and 159 million trophozoites on the 110th, 273d and 280th days, re-

spectively, and experienced a protracted subclinical parasitemia after the first reinoculation, a transitory parasitemia and one elevation of temperature immediately after the second, and a transitory parasitemia a few days after the third. When this patient was finally reinoculated by 45 demonstrably infected mosquitoes (a massive dose of sporozoites), trophozoites were again discernible for 5 days after a prepatent period of 13 days (Boyd and Kitchen, 1943).

Nevertheless we have tried to immunize with attenuated sporozoites. Recalling that the sporozoites in infected mosquitoes which have been stored at 4°C. for 50 or more days are degenerated and non-infectious, we attempted the immunization of patients with these presumably attenuated sporozoites, as shown in Table 2.

These patients were later inoculated in series, with controls, by virulent sporozoites of the same strain, as shown in Table 3.

It is not apparent that the application of the degenerated or attenuated sporozoites resulted in their recipients' exhibiting any discernible immunity to sporozites. It is obvious that the degenerated sporozoites inoculated probably have been few in numbers, while the test inoculations were probably done with an excessive number of virulent sporozoites. Perhaps the degenerated sporozoites introduced were insufficient to serve an antigenic function. Yet regardless of what might be the body's response to sporozoite antigen in large quantity, providing technical difficulties in the way of production could be overcome, it does not appear that the immunity manifest by a large proportion of the population living in highly endemic areas is likely to be attributable to an acquired immunity to sporozoites. The apparent limitation of the immune mechanism to a defense against trophozoites conceivably may be due to a difference in the antigenic composition of trophozoites and sporozoites, to limited numbers of sporozoites, or to the rapid sucession of the sporozoite stage by another, passed in a protected situation which does not permit them to function antigenically. On the other hand, the trophozoites, regardless of whether stemming from inoculation by sporozoites or trophozoites, are produced in sustained abundance in ample antigenic quantities, and occur in a situation where they are expected to exert their maximum capacity for stimulation.

The circumstance that through a series of reinoculations with the homologous living parasites, with or without the production of superinfections, a formidable degree of immunity is produced, leads one inevitably to query whether more or less comparable results might be secured from the inoculation of killed parasites. Consideration of this question immediately reveals the presence of technical obstacles. Among them may be mentioned: (a) the inability to secure adequate quantities of parasites from *in vitro* cultures, necessitating the employment of those present in fresh whole blood; (b) the circumstance that in the blood they are in intimate association with the erythrocytes; (c) the further circumstance that the parasitized erythrocytes are quite highly diluted among uninfected cells; (d) the difficulty of effectng a separation and concentration of the parasites from their host cells; and (e) the additional difficulty of killing the parasites without producing significant alteration of the cellular elements of the blood.

Since plasma quinacrine levels of 120 micrograms or thereabout are known to be capable of destroying parasites in the circulation, it appeared that the problem might

be tentatively approached by producing a high quinacrine level in test patients, into whom large parasite inoculations are made with the expectation that the quinacrine will quickly destroy the parasites *in vivo* permitting them to exercise whatever antigenic effect they may. Furthermore in this manner there is avoided the troublesome problem of killing and concentrating the parasites *in vitro*. It is well known that vivax infections induced by trophozoites are, in contrast with those induced by sporozoites, readily eradicated by plasmodicidal drugs (Boyd and Kitchen, 1946).

Two presumably infected patients were given 3 full therapeutic courses of quinacrine hydrochloride, receiving on the first day of each course a total of 0.4 gram, on the second day 0.6 gram, and for 10 days thereafter 0.3 gram until they had

FIG. 12. Patient B-3104. Showing result of natural inoculation subsequent to a series of immunizing inoculations effected by administration of living parasites while patient was saturated with quinacrine (*cf.* Chart 7, Fig. 2).

received in divided doses, a total of 4.0 grams. These courses were given in June, August and September. On the fourth day of each course the patients were intravenously inoculated with blood containing living *P. vivax* of the McCoy strain, amounting to 79 million and 109 million trophozoites for the first and second inoculations, and 263 million and 225 million, respectively, in the third. Parasites were seen in smears from both patients taken immediately after the first inoculation and on the following day; none were seen in either patient following the second inoculation, while one patient showed parasites on the day following the third inoculation. Apart from these instances, no parasites were noted, and the patients remained in good health. Quinacrine levels in both patients were in excess of 120 micrograms per liter on all occasions except the first inoculation of one, when it was between 80 and 120.

Obviously the test inoculation of these patients could not be effected until practi-

cally complete excretion of their accumulated quinacrine. In the following April their plasma gave determinations of 1.6 and 0.9 micrograms per liter, which might represent either a trace or a high blank reading.

The patient with 1.6 micrograms was inoculated with the McCoy strain by the application of two infected mosquitoes on April 2. Parasites were first seen in the smear of April 16, and an elevation of temperature on April 21 marked the onset of a series of 3 quotidian paroxysms, which terminated spontaneously (fig. 12).

The other patient was similarly inoculated on April 19 by a single infected mosquito. The smear of April 28 was the first to show parasites. The first elevation of temperature occurred on May 5, initiating a series of 7 quotidian paroxysms, which ceased spontaneously (fig. 13).

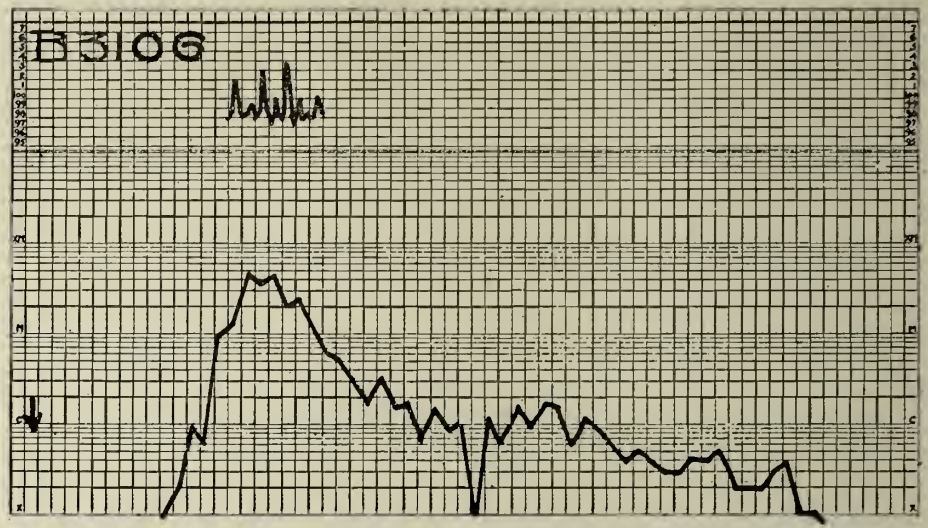

FIG. 13. Patient B-3106. Showing results of natural inoculation subsequent to a series of immunizing inoculations effected by administration of living parasites while patient was saturated with quinacrine (*cf.* Charts 7, 9, Figs. 2 and 3).

Assuming that these patients were originally fully susceptible they did not, on final inoculation with living sporozoites, prove refractory to inoculation, as would be expected in the case of a hyperimmune. Nevertheless the courses of their infections indicate that they were not completely susceptible, and they exhibited the following very definite characteristics of immunity: (a) the pyrogenic threshold was high; (b) the maximum density attained by the parasitemia was low, and (c) transitory, with (d) a rapid spontaneous decline; and (e) the clinical attack was short. From this it would appear that these patients have acquired an appreciable immunity following the intravenous administration of living parasites, effected at times when their plasma was saturated with quinacrine.

SUMMARY

The malarial histories of most neurosyphilitic patients referred for malaria therapy are unknown. Their response to inoculation with *P. vivax* by either natural or artificial means, permits of their classification in four categories:

(1) Inoculation resulting in a take: (a) an attack of three weeks or longer; (b) a short attack lasting from one day to two weeks or thereabouts; (c) without a clinical attack.

(2) Inoculation not resulting in a take.

An interpretation of the significance of these results is permitted from the homologous or heterologous reinoculation of patients on the service. Since infections of the character of 1 (a) have never been observed subsequent to reinoculation, it is inferred that patients with attacks of 3 weeks' duration or more were previously completely susceptible. Results like 1 (b) may follow either heterologous or homologous reinoculations, and in the latter case indicate that immunity is incomplete. Following homologous reinoculation, results like 1(c) or 2 may be secured. The latter indicates a very effective immunity, or a refractory condition.

It is readily made evident by reinoculation with demonstrably infectious material, that convalescents from vivax malaria have acquired, as a result of this experience, a distinct and at times very potent immunity to the homologous parasites. A comparison of the parasite density prevailing at the beginning and end of the clinical attack indicates that a tolerance to the presence of the parasites is the first manifestation of a change in the individual's status. The restraint in the exuberant multiplication of the parasites which is soon evident, is probably derived from the host, but is not likely specific. Some time after the infection becomes latent—and the latency may be regarded as an expression of the new state—the host becomes aggressively inhospitable to the trophozoites, being able to remove and destroy numbers fantastically greater than the scanty few which can initiate an infection in a susceptible person.

This mechanism appears to be directed against trophozoites, as reinoculation of an immune person with homologous trophozoites in blood may, in a refractory person, result in their immediate disappearance, while if the reinoculation is effected with infected mosquitoes, trophozoites will make their appearance after an appropriate prepatent period, but will soon disappear. Hence it does not seem to be operative against sporozoites.

Through repeated reinoculations with living homologous trophozoites and the production of superinfections, an individual may pass from stage 1(b) to stage 1(c) to stage 2 of complete refractoriness or hyperimmunity. Such a state appears to be of considerable duration, and it is not evident that its continuance depends upon a persistence of latent infection. Since attempts to passively immunize susceptibles through transfusions in which hyperimmunes served as donors have been failures, it is unlikely that the immunity is to any great degree humoral.

A few attempts to immunize with attenuated sporozoites have been unsuccessful, although it is realized that the possibilities have not been adequately explored. It does not appear that, even if possible, immunization to sporozoites is often if ever realized in nature, because of the relatively small amount of antigen available and introduced. Furthermore the available evidence indicates that the defense mechanism in hyperimmunes does not get into action until trophozoites are produced following mosquito inoculation. This suggests that either the sporozoites or their successors are inaccessible to the immune mechanism, or even perhaps that they are

of different antigenic composition, or that their numbers are insufficient to serve an antigenic function.

The possibility of effective active immunization with killed parasites is an intriguing subject, but it is beset by many technical difficulties. A simple approach, sufficient to determine its basic practicability, is afforded by inoculation of living parasites into susceptible subjects who have been saturated with quinacrine, thus killing the parasites *in vivo*. Two patients, originally presumed to be completely susceptible, who were given three inoculations of parasites when prepared in this fashion, reacted to the infection on subsequent reinoculation by infected mosquitoes as partial immunes of category 1(b).

RESUMEN

Las Historias maláricas de muchos pacientes neurosifilíticos en relación con la terapia de la malaria no se conocen. Su respuesta a la innoculación con *P. vivax* por medios naturales o artificiales permite su clasificación en cuatro categorías.

1) Inoculación con resultado positivo:
   a) Un ataque de tres semanas o más largo
   b) Un ataque corto que dura de una día a dos semanas, aproximadamente.
   c) Sin ataque clínico.
2) Inoculaciones con resultado negativo.

Una interpretación del significado de estos resultados se saca de reinoculaciones homólogas o heterólogas de los pacientes del servicio. Desde que las infecciones del carácter del número 1 a no se han observado nunca después de la reinoculación se deduce que los pacientes con ataques de 3 semanas de duración o más fueron previamente y por completo susceptibles. Resultados como el 1b pueden seguir bien sea a reinoculaciones homólogas o heterólogas y en el último caso indican que la inmunidad es incompleta. Después de reinoculación hemóloga se pueden obtener como 1c ó 2. El último indica una inmunidad muy efectiva o una condición refractaria.

Se ha hecho evidente por la reinoculación con material que ha sido demostrado infentante que los convalecientes de vivax malaria han adquirido, como resultado de esta experiencia una inmunidad distinta y en ocasiones muy potente para los parásitos homólogos. Una comparación de la densidad parasitaria prevalente al comienzo y al fin del ataque clínico indica que una tolerancia a la presencia de los parásitos es la primera manifestación de un cambio en el status individual. La restricción en la exuberante multiplicación de los parásitos que pronto es evidente, es probablemente derivada del huésped, pero no es específica, muy posiblemente. Algún tiempo después de que la infección se hace latente y la latencia puede ser mirada como la expresión de un nuevo estado, el huésped comienza agresivamente a ser inhóspito a los trofozoitos, siendo capáz de remover y destruir números fantásticamente mayores que los pocos que pueden iniciar una infección en una persona susceptible.

Este mecanismo aparece estar dirigido contra los trofozoitos puesto que la reinoculación de una persona inmune con trofozoitos homólogos en la sangre puede en una persona refractaria, resultar en la desaparición inmediata, mientras que si la reinoculación se efectúa con mosquitos infectados los trofozoitos hacen su aparición después de un período prepatente apropiado, pero también desaparecen pronto. Así pues no parece que es operativa contra los esporozoítos.

A traves de repetidas reinoculaciones con trofozoitos vivos homólogos y la producción de superinfecciones, un individuo puede pasar del estado 1b al estado 1c ó al estado 2 de completa refractariedad é hiperinmunidad. Tal estado aparece ser de considerable duración, y no es evidente que su continuidad depende de la persistencia de una infección latente. Desde que los ataques para inmunizar pasivamente los susceptibles al traves de transfusiones en las cuales se han usado hiperinmunes como donadores, ha tenido fallas, es poco probable que la inmunidad tenga a tal punto un grado humoral.

Unos pocos ataques para inmunizar con esporozoítos atenuados han dado resultado negativo, aunque se ve que las posibilidades no han sido exploradas de una manera adecuada. No se ve que auncuando posible, la inmunización a esporozoítos se realiza en la naturaleza, a causa de la relativamente poca cantidad de antígeno disponible é introducido. Además la evidencia que tenemos a la mano, indica que el mecanismo de defensa en los hiperinmunes no entra en acción hasta que los trofozoítos se producen después de una inoculación por un mosquito infectado. Esto sugiere que ó los esporozoítos o sus sucesores son inaccesibles al mecanismo inmune o posiblemente ellos tengan una composición antigénica diferente o que su número es insuficiente para completar una función-antigénica.

La posibilidad de efectuar inmunizaciones activas con parásitos muertos es un problema intrigante, pero está dificultado por muchos inconvenientes técnicos. Como un simple acercamiento suficiente para determinar su practicabilidad básica, se hace por inoculación de parásitos vivos en sujetos susceptibles que pueden estar saturados con quinacrine, matando así el parásito "in vivo." Dos pacientes, presumidos en un principio como completamente susceptibles, a quienes se les dieron 3 inoculaciones de parásitos con la técnica acabada de expresar, reaccionaron a la infección o a las reinoculaciones siguientes con mosquitos infectados, como inmunes parciales de la categoría 1b.

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SOME RECORDS OF MOSQUITO DISSECTIONS IN NORTHERN NEW GUINEA

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During the period from January 29 to June 18, 1945, mosquitoes were collected by members of the 19th Medical General Laboratory from several native villages in the vicinity of Hollandia, Netherlands New Guinea, and some of the collections were examined for malaria and filaria infections. Villages on the shores of Lake Sentani from which mosquitoes were collected included Swarekus, Poee, Simboro, Ajapo, Elim and Ase. Coastal villages near Hollandia which were included in the study were Nakasawa, Skosai, Holtekang Settlement, Engros and Tobadi. Besides the village collections, the study included a series of dissections of *Anopheles karwari* caught in a military camp in the Hollandia area.

Anopheles punctulatus punctulatus, *A. p. farauti* (formerly known as *moluccensis*) and intermediate forms of these were the only anophelines taken in the collections from the villages. Their relative prevalence is shown in Table 1. The intermediate form (the taxonomic status of which has not been fully determined) was the predominant one and constituted 74 per cent of the total. In the daytime collections of mosquitoes resting inside the houses, subspecies *punctulatus* was more numerous than *farauti*, while the night biting-collections (at one village only) gave about five times as many *farauti* as *punctulatus*. Specimens were identified as intermediate when the labium showed some white scales, usually a streak laterally and ventrally or a narrow ring, toward the apex, but not forming an extensive white ring involving most of the apical half, as found in subspecies *punctulatus*. All of the anophelines captured in daytime resting places inside the huts were either engorged or gravid.

EXAMINATIONS FOR MALARIA PARASITES

In the collections of *Anopheles punctulatus* from native villages, two sporozoite infections were found in a total of 186 gland examinations but no oöcysts were observed in 119 stomachs examined. Both specimens with sporozoites were among 52 of the intermediate form collected from Poee Village on Lake Sentani. This is the first record, known to the writers, of infection in this form, previous records having been referred either to subspecies *punctulatus* or *farauti* (*moluccensis*). Table 2 summarizes the examinations for malaria parasites.

A series of *Anopheles karwari* collected inside tents at night in a military camp near Hollandia[2] were examined for malaria infection. Of 119 examined, none

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[1] Under the direction of Col. Dwight M. Kuhns, M.C., Commanding Officer.

[2] King, Willard V. and Harry Hoogstraal, 1945. Three New Anopheline Records from New Guinea. J. Nat'l Malaria Soc. V, 153 June 1946.

contained sporozoites or oöcysts and only six showed ovarian development or engorgement with blood. Suppressive doses of atabrine were being administered to the troops in the area and there were no native villages within several miles of the location. For these reasons it is considered that opportunities for the mosquitoes to acquire infection in this area were slight.

EXAMINATIONS FOR FILARIA

A total of 203 specimens of the *punctulatus* complex were examined for filaria and 20 of these, or approximately ten per cent, were positive. Over half of the positives

TABLE 1

Relative Prevalence of Different Forms of Anopheles punctulatus *in Day and Night Catches in Native Villages*

FORM OF A. PUNCTULATUS	SWAREKUS VILLAGE		OTHER VILLAGES, DAYTIME ONLY	TOTALS
	Daytime catches	Night catches		
Subsp. *punctulatus*	5	13	46	64
Subsp. *farauti*	2	62	18	82
Intermediate form	26	170	213	409
Total	33	245	277	555

TABLE 2

Examinations for Malaria Parasites in Anopheles punctulatus *Collected from Villages*

FORM OF A. PUNCTULATUS	STOMACH		GLANDS	
	Total examined	Number positive	Total examined	Number positive
Subsp. *punctulatus*	19	0	12	0
Subsp. *farauti*	8	0	36	0
Intermediate form	92	0	138	2
Total	119	0	186	2

were found in the intermediate form. Advanced stages of filaria were observed in all three forms, indicating that the infection may be transmitted by any of them. Mature larvae (i.e. in the post-sausage-stage) were observed in seven specimens and one of these contained a larva in the labium. From several smears of stomach blood, the microfilariae present appeared to be *Wuchereria bancrofti*. The results of the dissections for filaria are shown in Table 3. With reference to Holtekang settlement, where the largest number of infected mosquitoes were obtained, more than half of the inhabitants were said to be evacuees from the island of Tarakan (off the Northeast coast of Borneo), and all except three of the dissected mosquitoes from this settlement were from a single bed net used by one of these evacuees.

TABLE 3

Filaria Infections in Anopheles punctulatus *Collected from Six Villages in the Hollandia Area*
(Examinations of thorax and head)

VILLAGE	INTERMEDIATE FORM		SUBSPECIES PUNCTULATUS		SUBSPECIES FARAUTI	
	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
Swarekus	26	1	5	0	2	0
Poee	56	4	10	1		
Skosai	42	1	10	0	4	1
Holtekang Settlement	18	5	10	2	7	5
Nakasawa	6	0	1	0		
Simboro	6	0				
Total	154	11	36	3	13	6
Per cent positive		7.1		8.3		46.2

TABLE 4

Filaria Infections in Armigeres obturbans Collected in Native Huts During Daytime

VILLAGE	STOMACH		THORAX AND HEAD	
	Total examined	Number positive	Total examined	Number positive
Poee	98	2	134	5
Swarekus	25	2	66	1
Others*	47	0	68	0
Total	170	4	268	6

* Elim, Ajapo, Ase and Simboro Villages.

TABLE 5

Dissections for Filaria of Miscellaneous Culicines from Tobadi, Swarekus and Skosai Villages

SPECIES	STOMACH BLOOD		THORAX AND HEAD	
	Total number examined	Number positive	Total number examined	Number positive
Culex siliens	13		13	
Culex fatigans	1		1	
Aedes aegypti	5		5	
Aedes (*Aedes*) sp	13	2	16	1
Aedes carmenti	1		1	
Aedes funereus	1		1	
Aedes ornatus	1		5	
Aedes scutellaris	1		1	
Mansonia uniformis	1		1	
Mansonia septempunctata	3		3	
Total	40	2	47	1

EXAMINATIONS OF CULICINES FOR FILARIA

The most abundant culicine represented was a species of *Armigeres*, possibly *obturbans* (Walker). The identity of this species is uncertain as males from the type locality (Amboyna, Moluccas) have not been described. Males collected in the native huts differ in genitalic characters from *A. milnensis* Lee, a common New Guinea species, and also from the so-called *obturbans* of India. The larvae of *?obturbans* were found breeding in large numbers in the native houses, in jars or urns containing a fermenting mixture of sago flour in water.

Female specimens of *Armigeres* collected during the daytime in native houses from six different villages were examined. Infected specimens were found in only two of the villages, Poee and Swarekus, and the infection rate for all examinations was low (2.2%). None of the infected mosquitoes contained advanced stages of the parasite, indicating that this species may not be an efficient vector in this area in spite of its high degree of domesticity. Results of these examinations are shown in Table 4.

Small numbers of other species of culicine mosquitoes collected from native villages were examined for filaria and the results are shown in Table 5. Infections were found in only one specimen of one species, *Aedes* (*Aedes*) sp., collected from Skosai Village. This specimen contained immature worms in the thorax. Two others had microfilariae in the stomach blood.

SUMMARY

Sporozoites of malaria parasites were found in the salivary glands of two specimens of the "intermediate" form of the *punctulatus* series of *Anopheles*, among 138 gland dissections of specimens of this form collected in native villages in the Hollandia area of northern New Guinea. This is the first record so far as known of infection in this form, which was the predominating one in these villages. Gland examinations of 12 specimens of subspecies *punctulatus* and 36 *farauti* (formerly known as *moluccensis*) were negative. No infections were found in a series of 119 *A. karwari* obtained from a military camp located several miles from any native village.

Filaria infections were found in the thoracic muscles or head of 20 or 203 specimens of the *punctulatus* series, for an infection rate of about ten per cent. Seven of the specimens (representing each of the three forms) contained matured larvae. Six individuals among 268 *Armigeres ?obturbans* and one among 13 *Aedes* (*Aedes*) sp. contained immature filariae in the thoracic muscles. Small numbers of nine other culicine species were negative.

RESUMEN

Esporozoítos de parásitos maláricos fueron encontrados en las glándulas salivares de dos ejemplares de la forma "intermediate" de la serie *punctulatus* de *Anopheles*, sobre un total de 138 disecciones de glándulas de ejemplares colectados en pequeñas poblaciones nativas en la área-Holandia de la parte norte de Nueva Guinea. Este es elprimer record conocido hasta ahora de infección en estaforma, que fué la pre-

dominante en estos villorrios. Exámenes de glándulas de 12 ejemplares de la subespecie *punctulatus* y 36 *farauti* (anteriormente conocida como *moluccensis*) fueron negativas. No se encontraron infecciones en una serie de 119 *A. karwari* obtenidos de un campo militar, localizado a varias millas de cualquier pueblecito de nativos.

Se encontraron infecciones filáricas en los músculos toráxicos de la cabeza de 20 entre 203 ejemplares de la serie *punctulatus*, con una rata de infección aproximadamente del 10 por ciento. Siete de los ejemplares (representadas cada una de las tres formas) contenían larvas maduras. Seis ejemplares de 268 *Armigeres obturbans* y 1 entre 13 de *Aedes* (*Aedes*) sp. contenían filarias inmaduras en los músculos toráxicos. Pequeños números disecados de otras 9 especies de culicinae fueron negativos.

TOXIC REACTIONS FOLLOWING ADMINISTRATION OF ATABRINE DIHYDROCHLORIDE

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Early in the War, the question arose as to whether the suppressive use of antimalarial drugs would interfere with the efficiency of Air Force personnel. The only drug available in quantity was atabrine and not much was known with regard either to its effectiveness as a suppressive or with regard to the toxic effects from its long continued use. At that time, the commonly accepted dose for suppression was 0.2 gram to be given on non-successive days twice a week. The common therapeutic dose was 0.1 gram three times a day for five days.

The first question to be settled was whether suppressive doses exerted deleterious effects at altitude. Some preliminary studies were done but these were greatly complicated by the fact that several of the relatively small number of subjects were made ill by the atabrine when it was given in this dose. Later, a study was conducted with enlisted personnel as subjects. A battery of tests was devised by the Department of Psychology and included addition tests, code, substitution tests, steadiness aiming tests, and compensatory pursuit tests. Each man had taken all of the tests in the high altitude chamber on at least ten previous occasions so that his performance was reasonably constant. Just before administration of the drug began, a control run was made at ground level and at 18,000 feet simulated altitude. On each run an equal number of men receiving atabrine and placebos were used. After the initial control run, each subject began taking either one tablet of atabrine dihydrochloride or a placebo tablet after each meal. At the end of five days another run was made in the high altitude chamber and the psychomotor tests were repeated. Each subject was provided with an oxygen mask and the tests were first performed with adequate oxygen and again 15 minutes after the oxygen had been turned off.

The tests employed were all those that had been demonstrated to be adversely effected by hypoxia. A careful statistical analysis of data revealed that there was no evidence that atabrine had any significant effect on performance either with or without added oxygen.

The next part of the study arose from the observations that the suppressive doses originally tried gave rise to gastrointestinal disturbances in several individuals, and, because early in 1942 there were conflicting reports from military channels concerning the toxicity of atabrine. Since the study began various doses have been tested along with placebos in several groups up until the autumn of 1944. The doses of particular importance are those in which 0.2 gram of atabrine was given twice a week on non-successive days and 0.1 gram of atabrine was given daily and 0.2 gram

[1] Now at the Department of Pharmacology, George Washington University School of Medicine.

was given at a single dose daily for the first week and 0.1 gram daily thereafter and when 0.5 gram was given at a single dose twice a week on non-successive days. This latter dose was employed as a control to determine whether a newer antimalarial drug gave rise to fewer gastrointestinal disturbances.

Records of mild reactions were kept but they were not reported since they did not seriously interfere with performance of duties and since they were almost as common among personnel receiving placebos as among those receiving atabrine. Any distinction between a moderate and a severe reaction is necessarily an arbitrary one but for the present purpose a moderate reaction was defined as one in which the individual was partially incapacitated for most of one day but able to resume full duties on the next day. A severe reaction was one in which the individual was incapacitated for one day or more, frequently with hospitalization. The toxic reactions usually consisted of diarrhea, nausea, vomiting and headache, or a combination of these. Less frequent reactions, some of them probably not due to atabrine administration, included vertigo, blurred vision, skin-disorders, insomnia, excessive sweating, pruritis, tinnitis, hyperpyrexia, and muscle and joint pains. Many of these symptoms were mild and were frequently the same as those reported by subjects receiving placebos. It should be remembered that the subjects employed were mostly medical officers attending the course for flight surgeons. As a whole they should be regarded as a highly suggestible group of subjects. This was reflected by a high percentage of reactions in the group receiving placebos. One man, for instance, was closely associated with some officers who were experiencing gastrointestinal disturbances from atabrine and, although he himself was receiving a placebo, he vomited eight times. When convinced that he was not receiving atabrine his concern shifted immediately from his physical to his mental condition.

The fever, muscle and joint pains were usually associated with other symptoms. In some instances temperatures of 102° to 103°F. were recorded upon admission to the hospital. There were two cases of mental depression and one other developed a mild anxiety state. In view of the psychoses that have since been reported in large groups receiving atabrine, it seems likely that these were early signs of the effects of atabrine on the central nervous system.

Coöperation of the subjects was excellent unless they experienced a moderate or severe reaction, after which many of them refused to continue with the experiment. In the group receiving 0.2 gram twice a week, due to the large number of toxic reactions, the number of subjects was reduced to less than one-half after the fourth dose. Some who continued the treatment nevertheless, had moderate to severe reactions and usually discontinued the drug upon the development of these reactions. Still others had no further symptoms throughout the experiment.

It is obvious from Figure 1 that far more reactions occurred with doses administered twice a week than with any other dose. It seems likely that this was due to one of several causes: The administration at one time of doses of 200 mgm. or more; the particular time interval chosen for administration of the drug; or a combination of these circumstances. The failure to observe many toxic reactions until the third dose with the groups receiving the drug twice a week, suggests that it may have been

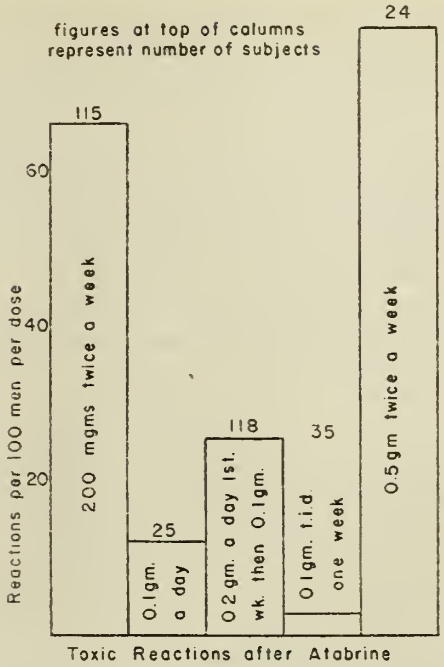

FIG. 1. Toxic reactions related to atabrine dosage

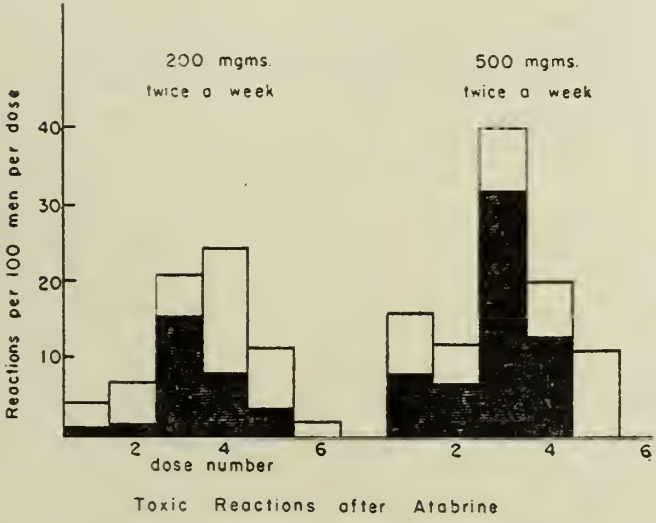

FIG. 2. Toxic reactions related to atabrine dosage

due to the distribution of the doses. Evidence that it was not due to taking as much as 0.2 grams on a single day was obtained from a group that were given the common therapeutic dose of 0.3 gram a day in divided doses. None of this group

had severe symptoms. More conclusive evidence was obtained in a group of men that were given 0.3 gram three times a day for a week in an attempt to analyze any electroencephalographic changes that might occur. Almost all of these men exhibited some signs of toxicity during the week and one man had questionable liver damage but in almost every case vomiting and diarrhea did not occur until the third or fourth day. Further evidence that the interval between doses was important was obtained in the group that received 0.5 gram twice a week. Although several exhibited toxic effects during the first and second dose by far the larger number became ill for the first time after the third or fourth dose.

Most of this data is now of mere academic interest since we observed and it is now amply confirmed by field studies that the administration of 0.1 gram of atabrine daily gives rise to very few gastrointestinal reactions.

SUMMARY

The administration of atabrine dihydrochloride in certain doses is associated with a high incidence of gastrointestinal activity.

The suppressive doses associated with the highest toxicity were those in which 0.2 gram or more were given as a single dose twice a week on non-consecutive days.

Toxic reactions were infrequent with daily doses of 0.1 gram.

RESUMEN

La administración de clorhidrato de Atabrina a ciertas dosis está asociada con una alta incidencia de actividad gastro-intestinal.

Las dosis supresivas asociadas con la mayor toxicidad fueron de 0.2 gramos o más, dadas en dosis simples dos veces por semana en días no consecutivos.

Las reacciones tóxicas, son infrecuentes con dosis diarias de 0.1 gramos.

A MALARIA RECONNAISSANCE OF THE REPUBLIC OF HAITI[1]

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GEOGRAPHY AND GEOLOGY

The Republic of Haiti occupies the western third of the second largest island of the West Indies—the other portion consituting the Dominican Republic. The island is situated between 18 and 20 degrees north latitude and between 71 and 74 degrees west longitude, approximately midway between Cuba and Puerto Rico. The maximum length of Haiti is about 180 miles; and the width along the Dominican border is 110 miles. Its land area, including the four largest island dependencies, is only slightly more than 10,000 square miles. This is due to the fact that the Gulf of Gonave almost bisects Haiti into two narrow peninsulas —the northwestern and the southwestern.

The country is chiefly mountainous, steep slopes rising directly from the coasts except at the infrequent intervals where wedges of plain extend into the mountain masses. Elevated plateaus as seen in Central and South America are not a feature of Haiti. The highest ranges attain up to 8,000 feet of altitude and consist for the most part of masses of limestone (1). They are frequently scarred by cliffs and intricately dissected by erosion.

Only one considerable river exists, the Artibonite with its large tributary, the Guayamouc. It is navigable for only the few miles where it is tidal. The total length of this river system is about 145 miles, and its watershed comprises almost one third of the country. A few short rivers in various parts of the Republic maintain a steady flow throughout the dry seasons; but the great majority of the streams are intermittent in character. Practically all of them are blocked at the mouth by bars of sand or gravel thrown up by wave and wind action, forming lagoons which offer ideal breeding places for anopheline mosquitoes. Since the principal human settlements are located along the coast in the vicinity of these streams, few are beyond the flight range of insects developing in these blocked outlets.

Haiti has several lakes and ponds. The largest of these, Etang Saumatre, has an area of 70 square miles, and its water closely resembles that of the sea in composition. Etang de Miragoane, the second largest, contains fresh water and discharges into the sea through a subterranean channel in a rocky ledge. The prolific breeding of mosquitoes along its grassy margins has been notorious since colonial times. Most of the other lakes and ponds are ephemeral and occupy sinkholes in limestone.

The geological nature of the island gives rise to many peculiarities of water dis-

[1] The observations outlined in this paper were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in cooperation with the Service National d'Hygiène Publique of Haiti.

tribution in addition to extensive underground drainage. Among these should be mentioned the appearance of innumerable springs and seepage areas at tidewater. These assume particular importance in the malaria picture, since coastal highways

TABLE 1

Mean Monthly and Annual Precipitation in Inches for Ten Towns of Haiti

TOWN	YEARS OF RECORD	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	MEAN ANNUAL RAINFALL
Port de Paix	30	5.1	3.4	2.5	2.5	3.5	3.2	2.5	3.4	4.6	4.9	8.4	5.0	49.0
Limbé	16	5.7	5.4	6.6	6.8	9.6	5.3	4.4	4.6	6.1	8.1	14.2	7.1	83.9
Cap Haitien	10	4.0	4.8	3.7	3.1	9.1	4.8	1.2	3.0	5.2	6.2	15.4	5.9	66.3
Gonaives	47	0.2	0.5	0.6	1.1	3.3	3.9	2.8	2.7	3.3	2.3	1.1	0.4	22.2
Mirebalais	14	1.3	2.2	3.9	9.1	11.6	13.0	12.4	15.4	15.2	11.9	5.6	2.1	103.5
Port-au-Prince	74	1.3	2.4	3.6	6.3	9.4	4.1	2.8	5.7	7.1	6.6	3.3	1.3	53.9
Jacmel	31	1.5	1.8	3.5	5.0	7.8	4.6	3.0	5.1	5.4	6.7	3.2	1.0	48.6
Petit-Goave	31	0.8	1.9	2.1	4.6	7.4	5.0	4.8	5.8	5.8	4.3	2.8	0.7	45.9
Cayes	32	3.0	3.4	4.3	7.1	11.7	7.0	4.9	8.7	9.0	14.5	7.0	2.6	83.3
Jeremie	31	2.7	3.1	3.3	3.9	6.1	4.4	3.5	3.9	4.0	5.4	6.8	3.5	50.6
Mean Annual Precipitation for Ten Stations														60.7

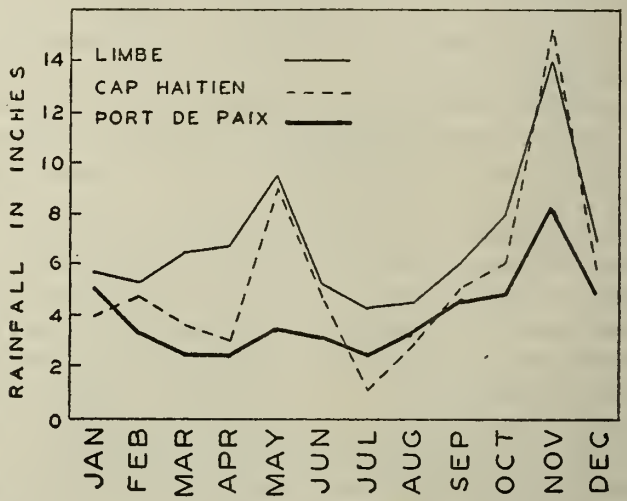

FIG. 1. MEAN MONTHLY PRECIPITATION FOR THREE TOWNS OF NORTH HAITI

have been built at beach level without sufficient provision for carrying this perennial water to the sea. One therefore finds in many localities a continuous mosquito breeding area, miles in extent, along the shoreward side of the highways. The same situation develops where roads are built across irrigated alluvial plains and act as unintentional dams to the runoff of surplus irrigation water.

CLIMATE AND RAINFALL

Haiti has a warm and notably equable climate. Temperature records do not exist except for a few towns situated at or near sea level, where annual means vary from 77 to 81 degrees Fahrenheit. There is usually a difference of about 10 degrees between the highest and lowest mean monthly temperatures, which is only about half the value of the mean diurnal difference between maximum and minimum. A few observations made at points above sea level would indicate that there is a drop of approximately 4 degrees Fahrenheit for each thousand feet of increasing altitude. Ice and frost have never been reported, even on the highest peaks.

Unlike the relatively uniform temperatures encountered in various parts of the Republic, the rainfall shows great irregularites in its distribution by season and locality. This is to be expected from the mountainous nature of the island and from the influence of the northeast tradewinds. There is hardly a region of the country where one may not travel in a few miles from a humid to an arid climate zone. This effect is very apparent when viewing the country from an airplane when extreme contrasts in types of vegetation are displayed below.

Local variations in precipitation being so characteristic, it will suffice to say that for the majority of stations there are two well-defined rainy seasons. One occurs in the spring; and the other in the fall—dates varying from place to place and from year to year. In these zones the winter dry season lasts from December to March; while the summer one is rarely so pronounced and is generally of but one month's duration. Table 1 summarizes the monthly and total annual rainfall for ten stations in widely separated areas of the Republic (2). Figure 1 plots the data for the first three towns of the list and shows the May and November maxima. Towns with low total annual rainfall usually show a single long season of precipitation, extending from April to November—the balance of the year being practically devoid of rain. Towns with very high total values likewise fail to show a pronounced summer dry season.

Another characteristic of the precipitation is that it falls in sudden storms; so that a major part of a month's rainfall may be accounted for in a few rains of only a few hours' duration. However, along the north coast and on the northern edge of the southwestern peninsula, winter precipitation may take the form of a light drizzle that continues for days at a time.

Violent storms are usually confined to the drier regions of the Republic. On infrequent occasions, destructive hurricanes have swept the south coast. Hail is sometimes reported as an accompaniment of summer thunderstorms.

Observations on relative humidity are lacking except for the station at Port-au-Prince, the capital. Here, the yearly average is about 70 per cent, with monthly averages ranging from 63 to 75 per cent (3). Daily fluctuations are more marked, a range of 30 being normal between a high of about 80 per cent at midnight and about 50 per cent at noon. Haiti thus has a less oppressive climate than that found in many other countries with the same temperature levels. The great regularity of the daily land and sea breezes throughout most of the year also mitigates the tropical heat in almost all the towns of the Republic.

VEGETATION AND AGRICULTURE

Exploitation of the forests for their commercially valuable woods since early colonial times and the clearing of mountainsides for the planting of coffee and food crops has left only isolated small areas of the original tropical rainforest. There remain a few stands of virgin timber, including pine, on the less accessible slopes of the higher mountain chains. Extensive areas once heavily wooded are no longer under cultivation and are now covered with brushwood or grass. The foothills and watered plains rarely display trees other than the usual tropical fruit-bearing varieties, though an occasional giant silk-cotton tree or a clump of useful palms lends interest to the landscape.

The arid plains and southwest slopes of most mountains are covered with xerophytic vegetation including fine examples of arborescent forms of cactus. Acacias, aloes, agaves, palmettos, and trees of the mesquite type fill in the picture in these forbidding regions. Where these dry plains approach a zone which is more abundantly watered there develops an area of grassy savanna which is utilized for grazing.

Salt flats occur where a coastal plain receives a meagre rainfall. In the tidal zones springs and lines of seepage usually encourage more or less extensive mangrove swamps. Prolific breeding of the malaria vector occurs in the brackish water of these swamps, especially during the rainy season or when swathes are cut for collecting tanning bark or firewood.

Areas utilized for the growing of export crops such as coffee, sugar, cotton, and bananas comprise a far smaller proportion of the total arable land than is the case in the neighboring islands of Cuba, Puerto Rico, or Jamaica. The principal crops grown for domestic consumption are corn, Indian millet, rice, peas, and beans. These along with cassava, sweet-potatoes, yams, breadfruit, and plantains make up the bulk of the local diet. Green and yellow vegetables are notably scarce; but this deficiency is no doubt made up to some extent by the prodigious consumption of mangoes, sour oranges, alligator pears, and a host of other tropical fruits in season.

Animal husbandry is little developed on the island, each household keeping its own chickens, pigs, and goats. Cattle are seldom seen in herds and are generally driven into the towns for slaughter. Dairying, as we know it, is practically unknown. Fishing is nowhere practiced on a considerable scale, although it is often possible to secure some fresh fish from the numerous canoe landings along the coast.

Methods of farming are very crude, the use of the plough and fertilizer being unknown except in the few sections of the irrigated plains devoted to the growth of export crops. This is to be expected where the average farm is seldom more than one or two acres in extent, and where even the steepest hillsides are planted with grain crops.

THE POPULATION

The aboriginal Indians of the island of Haiti, estimated at over one million in the time of Columbus, were quickly decimated by the early exploiters of the new land. In the course of succeeding centuries these were replaced by slaves from Africa and their descendants. At no time in its history has Haiti had a very large proportion of

inhabitants of white origin. Today the population is largely made up of negroes, a certain fraction of whom show evidence of mixed ancestry. The small component of white and Asiatic race groups is confined to the more important commercial centers.

No census has been taken in modern times, and present unofficial estimates range from two to three million people. Having had occasion to visit every corner of the country during the present reconnaissance, we are under the impression that the former figure is closer to the truth. Any generalizations as to the density of population would prove misleading as the people are very unevenly distributed. The irrigated plains and coffee-growing mountain slopes are very densely inhabited—an estimate of 300 to 400 persons per square mile being undoubtedly conservative. Relatively large geographic areas we have visited support a very sparse population.

Port-au-Prince, the capital, is the only large city in Haiti. Its population has likewise never been counted; but must lie somewhere between 100,000 and 200,000. The total combined population of the other large towns would probably not exceed even the lower of these figures; so that it would be safe to say that one half of the strictly urban residents live in this one city. From the standpoint of malaria control, the inhabitants of the 10 or 15 towns of from 1,000 to 5,000 population live under approximately the same conditions as the general rural fraction of the people who make up about 85 per cent of the whole.

A fact of considerable importance in its relation to the distribution of malaria is that there is a tremendous influx of mountain folk into the coastal market centers at relatively constant intervals throughout the year. Each roadside or village market has its special "day" of the week when visitors from a wide surrounding area may outnumber its permanent population many fold. It is unfortunately a common custom of those residing in the more remote hill districts to arrive at about sundown before market day, sleeping in the open with their produce where they are exposed to the bite of infected anophelines which have found shelter in neighboring huts. These persons thus maintain a relatively constant supply of gametocytes in their home settlements, where mosquito density may never be very high or where anopheline breeding is strictly confined to the rainy season.

ECONOMIC DEVELOPMENT

As previously stated the economy of Haiti is purely agricultural. There are no mining or industrial interests of consequence except for one or two large sugar mills. The type of agricultural product exported varies considerably from year to year, depending chiefly on world prices. Coffee is perennially important; while sugar, cotton, grains, cocoa, and dye-woods appear and disappear from the export list in turn. Imports vary less in kind but greatly in volume from year to year. Most important are cotton fabrics, petroleum products, soap, hardware, salt fish, and flour. During late years the dollar value of motor vehicles imported has increased considerably.

VITAL STATISTICS

The usefulness of available mortality and morbidity records for purposes of the

present survey is questionable. Data are collected from relatively few areas of the country, and laboratory confirmation of reported disease is an exceptional event. No attempt is therefore made to tabulate this material.

Efforts made to plot reported annual malaria deaths or cases against such factors as total rainfall have failed to show any significant correlation. The records of private physicians have been useful in only a few rare instances and do not lend themselves to analysis in a general reconnaissance. Patients are rarely accepted for treatment of malaria in the public hospitals, so that hospital records do not generally contribute a fair indication of seasonal or relative incidence of the disease.

PREVIOUS MALARIA INVESTIGATIONS

Local malaria surveys were made from time to time during the period of American occupation. A more detailed assessment of the sanitary situation was made by a group of investigators during 1924 and 1925, when laborers were being recruited from Haiti for work in neighboring islands. The clinical and epidemiological phases of the malaria section were handled by Dr. G. C. Payne, with Dr. W. A. Hoffman contributing entomological data. This material was never published, but some of it appeared in the annual reports of the Director-General of the Service d'Hygiene (5). However, this work was carried on in only a few scattered areas, and made but a limited contribution to the general malaria picture.

For the past several years it had become increasingly apparent to the Haitian Government that a serious and continued effort should be made to control the ravages of this disease which so greatly reduces the efficiency of local labor. An invitation was therefore extended to The Rockefeller Foundation to organize and conduct a nationwide survey so as to orient properly the planned program of malaria abatement.

METHODS OF THE MALARIA RECONNAISSANCE

During the early months of 1940 arrangements were completed between the Service National d'Hygiene of Haiti and The Rockefeller Foundation to conduct a malaria survey of the whole Republic. The former organization supplied all personnel and paid for all expenses in the field. The director of the survey, a staff member of the International Health Division, and the Haitian microscopist were supplied by The Rockefeller Foundation, which also furnished equipment and supplies for the laboratory examination of blood slides.

As has been the case in several other malaria surveys in this region of the world, it was decided to limit the field work to an examination of school children (6, 7, 8, 9). This method is generally considered to give a fair representation of the malaria incidence in a community; but there are several factors to be borne in mind in the interpretation of results—especially as they apply to Haiti. The children in the numerous rural schools come from considerable distances and thus represent conditions over relatively large areas. The topography of the island is such that many altitude levels may be included in a single group of pupils. Again, those individuals who were absent from school because of illness at the time of the visit are not represented in the sample. Many of these in certain zones were clinically sick with

malaria. Finally, the crowded schedule of field work precluded a careful check on the previous places of residence of each child, so that occasionally a malaria infection may be incorrectly attributed to a given locality.

The plan adopted for the collection of field data was to examine all school children for splenomegaly, and a selected group of them for parasites in the blood. All individuals showing enlargement of the spleen were included in the blood survey, whereas only 25 per cent of those with normal spleens were so tested. It was necessary to limit the blood collections in this way as only one microscopist was available at the central laboratory. In the case of those villages with less than 50 school children, all were examined for parasites. For the larger cities—where most children attend school—it was considered sufficient to record data for 50 individuals from each school until at least 2 per cent of the total estimated population of the town was represented.

The routine of a visit was to a large extent standardized. Thus, upon entering a school, one of the two members of a team took his place near the doorway of an empty examining room and set out his equipment for making blood smears. He then recorded on a special form the name, age, and sex of each child as it was sent into the room by the teacher. The subject then proceeded to the second physician for spleen palpation. The child was placed on his back on a bench or low table with his knees and thighs flexed to obtain relaxation of abdominal muscles, and his clothing was displaced to expose the lower part of the chest and the whole abdomen. The examination for splenic enlargement having been made, the results were called out to the teammate who recorded it against the child's name according to the classification as described by Boyd (4). In this method, P.D.I. represents those spleens palpable only on deep inspiration and numerals I, II, III, and IV represent increasing sizes, the last group having spleen borders palpable below the umbilicus. Each child showing any degree of splenic enlargement returned at once to the first observer who made a thick drop and a thin blood smear on the same slide. The serial number was inscribed on the thin smear at the same time as it was entered against the child's name on the sheet. Those whose spleens were not palpable also filed past the recorder on their way from the room and it was his duty to make smears on every fourth child against whom he recorded a "negative" spleen result. In cases where complete relaxation of the abdominal muscles was not obtained by the examiner the pupil was dismissed and considered as not examined. This routine largely eliminated the possibility of errors in recording and slide numbering. Each team was assigned an unbroken series of 5,000 serial numbers to avoid possible confusion in the laboratory, where many sets of slides might arrive simultaneously from the two units.

The slides, accompanied by the name lists, were dispatched by any available means of transport so as to arrive at the laboratory before five days had elapsed since collection. This was necessary to secure uniform dehemoglobinization of the thick smears, especially those from the hot and dry regions of the country. Staining was completed immediately upon receipt, and the slides were filed for study in the order of their arrival.

Every effort was made to achieve uniformity in staining technique throughout the survey. This was to a large extent possible in spite of initial difficulties in securing a good quality of Giemsa stain. Following the handling of the first 600 slides (all from Port-au-Prince) we obtained a large enough batch of a satisfactory Harleco product to last until the end of the study. Trials with various dilutions and staining times and a standardization of treatment for the distilled water for hydrogen ion concentration led to a routine that gave fairly uniform results. With the exception of the first few hundred slides of the Port-au-Prince series, all specimens were reported upon by a single microscopist, Mr. Ferdinand Vital. As an additional check on the reporting of results, 500 slides were later selected at random and dispatched to the Station for Malaria Research under the direction of Dr. Mark F. Boyd. Discrepancies disclosed by this check were of a minor nature and were concerned chiefly with the species identification of specimens showing only young ring forms. Our laboratory was found to have classified about 12 per cent of these positive slides as *P. falciparum* where the Florida laboratory reserved judgment. In view of the fact that this species accounted for over 85 per cent of the total infections discovered, the final result would not have been greatly affected by classifying these somewhat doubtful specimens as of undetermined species.

During the first three weeks of the study, while working on slides from the capital, examination of the thick smears was carried on for 10 minutes before a negative result was recorded. This period was then shortened to five minutes without a significant number of positives being missed. Additional time was taken on positive slides to examine the thin smear where species diagnosis could not be made with reasonable certainty of the forms encountered in the drop. No attempt was made to estimate the relative abundance of parasites, and careful search for gametocytes was early abandoned.

School visits were commenced late in October, 1940, and were completed in January, 1942. The first two months were devoted to the training of the four physicians in surveys of the schools of the capital. Frequent checks were made on spleen reports by one of the authors, and on many occasions two or more men examined the same children independently, later comparing findings. As a result of this preliminary work it is felt that a certain degree of uniformity was achieved in reporting splenic indices from various parts of the country. At the termination of the Port-au-Prince survey, two teams were organized and each was assigned to a large geographical area. As the two school terms coincided with the periods of most active malaria transmission for most sections of the country, it is quite possible that higher parasite rates may have been encountered than would have been the case had house-to-house visits been made throughout the calendar year.

Each team was required to search the area surrounding a school for breeding places for anopheline mosquitoes. Larvae and pupae were collected in preservative and dispatched to the laboratory for identification. No attempt was made to record the density of larvae in a given water deposit, but a brief description of the breeding place and the conditions under which the collection was made were recorded in a special field book. The time taken for these searches varied from place to place. Also, it must be borne in mind that most localities were only visited once, so that no

larvae are reported from several places with high malaria rates. This was usually the case when the visit followed a heavy rainstorm, or when the town was studied during the long dry season.

Field data and laboratory results were transferred in the central office to specially printed punched cards similar to those used by Boyd in Jamaica (6). These were inscribed with the name of the pupil and identification data relating to the school. Holes were available for sex, age group, political subdivision, altitude of school, size of spleen, presence or absence of blood parasites, and the species of plasmodium encountered. A special punched hole was reserved for the rare case considered abnormal in some respect, this making it an easy matter to set aside the card during later analysis where its inclusion might introduce an avoidable error. Thus, the infinitesimal number of white children examined did not contribute data to the final analyses as presented in the tables.

TABLE 2

Analysis of Population Sample Examined—School Children of Haiti, 1940–42

(Age and Sex Distribution)

AGE	MALES	FEMALES	TOTAL
0–4	444	391	835
5–6	1,996	1,593	3,589
7–8	3,807	2,870	6,677
9–10	5,273	3,749	9,022
11–12	5,983	3,426	9,409
12+	1,239	514	1,753
Total.....	18,742	12,543	31,285

RESULTS OF THE RECONNAISSANCE

Examinations of 31,285 school children in 826 primary schools were made to determine malaria incidence in all parts of Haiti. The study included all primary schools except 21 which could not be located or were too small to warrant the effort to reach them. Eight of these are known to be situated in the high mountains, far removed from potential anopheles breeding areas. Thus, about 98 per cent of the schools listed appear in our records. In the absence of census data of any kind it is difficult to estimate what percentage of the total population in the 4 to 14 year age group attend school. It would hardly exceed 15 to 20 per cent in any case.

Class attendance at the time of our visits varied considerably from place to place. Schools in the five larger cities were found to have from 70 to 85 per cent attendance on the day of examination; while in some of the rural areas the figure dropped to as low as 25 to 35 per cent. This was generally the case where planting or harvesting of crops was in progress during the school year. We were unable to estimate closely the number of absences due to illness, but members of the survey teams report it to be a minor factor.

Table 2 gives an analysis of the school population examined by sex and age.

TABLE 3

Splenic Index by Departement, Arrondissement, and Commune—Schools of Haiti

ARRONDISSEMENT	COMMUNE	SCHOOLS VISITED	SPLENIC INDEX
	Departement de l'Artibonite		
Dessalines	Dessalines	6	3.7
	Grande Saline	6	3.7
	Petite Riviere	10	7.9
		22	5.4
Gonaives	Anse Rouge	6	2.5
	Ennery	4	13.0
	Gonaives	24	2.2
	Gros Morne	14	14.9
	Terre Neuve	8	0.3
		56	5.7
Hinche	Cerca Carvajal	2	35.0
	Hinche	9	22.5
	Maissade	6	78.9
	Thomonde	2	68.7
	Thomassique	1	7.7
		20	43.3
Saint Marc	La Chapelle	3	40.6
	Saint Marc	19	5.7
	Verrettes	8	15.3
		30	11.1
Marmelade	Marmelade	2	2.0
	Saint Michel	10	10.2
		12	9.0
Total for Departement.....		140	12.6
	Departement du Nord-Ouest		
M. St. Nicolas	Baie de Henne	3	2.0
	Bombardopolis	3	4.3
	Jean Rabel	12	31.4
	M. St. Nicolas	6	56.5
		24	27.8
Port de Paix	Anse a Foleur	4	32.5
	Bassin Bleu	5	1.7
	La Tortue	5	5.2

TABLE 3—*Continued*

ARRONDISSEMENT	COMMUNE	SCHOOLS VISITED	SPLENIC INDEX
Departement du Nord-Ouest—*Continued*			
Port de Paix—*Cont.*	Port de Paix	13	13.0
	St. Louis du N.	6	12.3
		33	11.6
Total for Departement		57	19.4
Departement de l'Ouest			
Jacmel	Bainet	12	4.9
	Cayes-Jacmel	4	3.7
	Cotes de Fer	7	15.8
	Jacmel	25	15.8
	Marigot	3	7.8
	Trouin	3	4.1
		54	11.5
Las Cahobas	Belladere	3	42.7
	Las Cahobas	10	21.7
		13	27.9
Leogane	Grand Goave	6	12.8
	Leogane	9	26.8
	Petit Goave	18	41.4
		33	32.3
Mirebalais	Mirebalais	9	39.0
	Saut d'Eau	5	42.8
		14	40.2
Port au Prince	Arcahaie	9	5.4
	Cabaret	1	0.0
	C. des Bouquets	10	4.7
	Ganthier	9	4.8
	Gressier	1	31.4
	La Gonave	5	18.7
	Petionville	5	1.5
	Port au Prince	47	4.3
	Thomazeau	2	13.6
		89	5.2
Saltrou	Anse a Pitre	2	33.8
	Grand Gosier	7	4.7
	Saltrou	4	29.0
		13	17.9
Total for Departement		216	13.4

TABLE 3—*Continued*

ARRONDISSEMENT	COMMUNE	SCHOOLS VISITED	SPLENIC INDEX
	Departement du Nord		
Borgne	Borgne	8	19.1
	Port Margot	10	18.1
		18	18.7
Cap Haitien	Acul du Nord	7	26.3
	Cap Haitien	35	15.3
	Limonade	3	31.3
	Milot	3	14.0
	Plaine du Nord	6	44.8
	Quartier Morin	6	23.0
		60	21.1
Fort Liberte	Acul Samedi	2	16.4
	Fort Liberte	3	64.5
	Grand Bassin	2	40.6
	Les Perches	4	12.6
	Mont Organise	1	2.2
	Ouanaminthe	11	33.7
		23	34.1
Grande Riviere	Bahon	5	19.0
	Dondon	6	8.1
	Grande Riviere	14	19.4
	La Victoire	2	28.6
	Pignon	2	8.7
	Ranquitte	3	1.2
	St. Raphael	2	27.0
		34	16.0
Limbe	Limbe	13	25.6
Plaisance	Pilate	7	0.4
	Plaisance	10	1.8
		17	1.4
Trou	Caracol	2	67.2
	Ste. Suzanne	3	10.1
	Terrier Rouge	5	56.9
	Trou	6	30.5
		16	37.9

TABLE 3—*Continued*

ARRONDISSEMENT	COMMUNE	SCHOOLS VISITED	SPLENIC INDEX
	Departement du Nord—*Continued*		
Vallieres	Carice	2	6.5
	Cerca la Source	7	3.2
	Mombin Crochu	2	1.6
	Vallieres	4	9.3
		15	5.4
Total for Departement.....		196	20.4
	Departement du Sud		
Aquin	Aquin	14	35.7
	Cavaillon	8	44.1
	St. Louis du Sud	4	81.8
		26	43.3
Cayes	Camp Perrin	5	9.8
	Cayes	37	36.6
	Chantal	2	29.5
	Port Salut	5	20.2
	St. Jean du Sud	9	32.8
	Torbeck	4	35.1
		62	31.1
Coteaux	Chardonnieres	5	25.5
	Coteaux	4	43.5
	Les Anglais	3	52.8
	Port a Piment	6	36.6
	Roche a Bateau	5	19.6
		23	34.2
Grand'Anse	Abricots	3	42.9
	Corail	8	17.4
	Jeremie	29	5.2
	Moron	3	16.4
	Pestel	3	4.2
	Roseaux	8	13.1
	Trou Bonbon	4	17.0
		58	10.0
Nippes	Anse a Veau	10	35.5
	Asile	3	48.9
	Baraderes	2	83.7
	Grand Boucan	1	26.7

TABLE 3—*Continued*

ARRONDISSEMENT	COMMUNE	SCHOOLS VISITED	SPLENIC INDEX
Departement du Sud—*Continued*			
Nippes—*Cont.*	Miragoane	9	12.3
	Petite Riviere	4	42.6
	Petit Trou	3	76.0
		32	36.9
Tiburon	Anse d'Hainault	4	22.5
	La Cahouanne	1	56.7
	Dame Marie	7	34.6
	Les Irois	2	45.2
	Tiburon	2	38.4
		16	34.4
Total for Departement.....		217	28.2

Of a total of 31,285 children, 18,742 were boys and 12,543 were girls. Enrollment of girls drops off in the 11- to 12-year age groups, while the boys usually continue with their schooling for one or two years longer.

Table 3 presents a condensed summary of spleen indices for all 826 schools, grouped by major and minor civil administrative divisions of the country. The "department" corresponds roughly to a state or province in other countries. The "arrondissement" is also a well-defined area which is ordinarily named from its chief town. It represents what is known as a county or borough in the United States or as a municipio in Latin America. The "commune" is rather less well defined and corresponds to some extent to an incorporated township. The area comprising this minor division varies enormously from place to place and the total number of communes is constantly subject to change. In cases where the survey teams were unable with certainty to determine in which commune a school was located, it was classified along with the schools of the town to which the population of the area usually repaired on market-day.

The lowest general spleenindex for a departement was 12.6 per cent in Artibonite; the highest was 28.2 per cent for Departement du Sud. Greater variation was encountered among the different arrondissements, as would be expected. Plaisance, situated well inland in a mountain valley gave a 1.4 per cent rate; while the highest rate, 43.3 per cent was shared by Aquin on the south coast and Hinche in the upper plains of the Guayamouc River. Commune rates varied from less than 1 per cent at Pilate, Terre Neuve, and Cabaret to over 83.7 per cent at Baraderes, situated in a swamp on the north coast of the southern peninsula.

Table 4 shows a significant correlation between school spleen rates and altitude when all schools in the country are grouped together in zones of 1,000 feet. Smaller distinctions in altitude give rise to a less clear picture, since as a general rule children

in many instances walk down the hillsides to attend schools situated on the main highways. Even in the case of the capital, there is a constant interchange of pupils with its largest suburb, Petionville, situated at 1,300 feet behind the city. From the very topography of the country, a few hundred feet of altitude does not ordinarily represent a great linear distance, and many elevations are included in the flight-range of anophelines from a single breeding area. Figure 2 shows in rough outline the altitude zones of the country; while Figure 3 indicates the spleen rates by communes. High rates were recorded from schools in the 1,000 to 2,000 foot zone only in the area of the great central plains.

Spleen rates have been tabulated according to age groups in table 5, but no significant difference can be established between one group and another. This table also presents a tabulation of spleen palpations according to the size of spleen recorded and gives the calculated "average spleen" for the six age groups. The fact that the highest spleen rate as well as the largest "average spleen" are to be found

TABLE 4

Spleen Enlargement, by Altitude Zones

Haiti, 1940–42

ALTITUDE	NUMBER OF CHILDREN EXAMINED	NUMBER WITH ENLARGED SPLEENS	SPLEEN RATE
feet			*per cent*
0 to 999	22,427	4,594	29.8
1,000 to 1,999	7,220	974	13.5
Over 2,000	1,638	101	6.2
Total.....	31,285	5,769	18.4

in the youngest age group examined might indicate that these children are the most severely infected group.

For every age group it may be stated that the larger the spleen the less frequently was it encountered; so that a fairly uniform curve of decreasing frequency can be plotted for each group. In this respect our results are at variance with those reported by Downs, Gillette, and Shannon for negroes in Trinidad (15). They found almost twice as many children in the size II group as in size I or the P.D.I. classification.

Table 6 displays the survey results so as to show the association of positive blood parasite findings with varying frequencies of enlarged spleens encountered in the different geographical areas of the Republic. The twenty-seven arrondissements have been ranked in decreasing incidence of splenomegaly (43 to 1 per cent) with corresponding parasite rates given for both the spleen-normal and spleen-enlarged group. It is quite apparent from the table that the higher the splenic index of an area, the higher the incidence of parasitemia both in those with normal spleens and in those with splenomegaly.

This table also shows the adjusted general parasite rate for each arrondissement. This figure is computed by weighting the percentage of blood positives found in the

FIG. 2. ALTITUDE ZONES

FIG. 3. RESULTS OF SPLEEN EXAMINATIONS

spleen-normal sample as though all children palpated were examined for parasites. So adjusted, the table shows that as a measure of malariousness in a country like Haiti the efficiency of the method of spleen palpation is amply confirmed by the results of our blood survey.

Closer inspection of Table 6 shows that as a general rule the adjusted parasite rates found are considerably higher than the spleen rates for a given locality—sometimes twice or even three times as high. The explanation for this was apparent in the case of some districts like Borgne or Grande Riviére, where our visits coincided with a local epidemic. In these two towns, for instance, there was a tremendous flux of the population following the recent border disturbances and a recruitment of mountain folk to work on the war-inspired rubber and sisal plantations of the north coast.

Another factor which contributed to the disparity of the two indices in many

TABLE 5

Splenomegaly by Age Groups and Degree of Splenic Enlargement

General Summary, Haiti, 1940–42

AGE	NUMBER EXAMINED	SPLEEN P.D.I.		SPLEEN I		SPLEEN II		SPLEEN III		SPLEEN IV		TOTAL WITH ENLARGED SPLEEN		AVERAGE SPLEEN
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
0–4	835	69	8.2	59	7.1	36	4.3	4	0.5	4	0.50	172	20.6	0.40
5–6	3,589	251	7.0	234	6.5	125	3.5	30	0.8	3	0.05	643	17.9	0.34
7–8	6,677	529	7.9	374	5.4	198	2.9	46	0.7	4	0.10	1,151	17.2	0.31
9–10	9,022	830	9.2	513	5.9	269	2.3	46	0.5	3	0.03	1,661	18.4	0.32
11–12	9,409	937	9.9	579	6.3	237	2.5	44	0.4	3	0.03	1,800	19.1	0.32
12+	1,753	197	11.3	93	5.3	41	2.9	10	0.5	1	0.06	342	19.5	0.31
Total....	31,285	2,813	9.0	1,852	6.0	906	2.8	180	0.6	18	0.05	5,769	18.4	0.32

sections was that the school year, during which all field work was done, comprised most of the months of heaviest local anopheline production. However, it is most likely that the relatively low spleen rates encountered generally were due to the recognized fact that spleen enlargement in malaria is not so apparent in the negro as in other races.

The positive correlation between the frequency of parasitemia and the increasing sizes of spleens encountered is apparent from Table 7. For the country as a whole, children with normal spleens gave a 21 per cent positive blood-smear result; but variations from this figure were notable from place to place. The group showing the least degree of splenic enlargement, in contrast, showed 68 per cent parasitemia, with figures of 78, 80, 80, and 100 per cent for the ascending scale of sizes. The No. IV spleen group was unexpectedly small—only 18 children of 5,769 with enlarged spleens having been found with the spleen extending below the umbilicus. Owing to the very small sample in this group, it is probable that we are not safe in assuming that No. IV spleens are invariably associated with parasitemia. In fact,

other surveys have brought out a slight dropping off in the parasite rate in the No. III and No. IV classifications (6, 7, 10). In a survey of El Salvador reported by

TABLE 6

Association of Parasite Findings with Spleen Negative and Spleen Positive Groups, Showing Parasite Rates Adjusted for Method of Sampling

By Geographic Area, Haiti, 1940–42

ARRONDISSEMENTS	TOTAL WITH SPLEEN EXAMINATIONS			TOTAL EXAMINED FOR PARASITES						ADJUSTED PARASITE RATE
				Spleens normal			Spleens enlarged			
	Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive	
										per cent
Aquin	891	386	43	121	49	41	386	315	82	58
Hinche	805	349	43	105	58	55	349	280	80	66
Mirebalais	515	207	40	74	30	41	207	160	77	55
Trou	567	215	38	79	36	46	215	182	85	60
Nippes	805	297	37	115	48	42	297	232	78	55
Tiburon	479	165	34	76	17	22	165	100	61	36
Coteaux	849	290	34	133	47	35	290	228	79	50
Fort Liberté	744	254	34	116	37	32	254	188	74	46
Léogane	1,504	486	32	246	119	48	486	402	83	59
Cayes	2,149	669	31	342	111	33	669	489	73	45
Lascahobas	377	105	28	62	10	16	105	63	60	28
Mole St. Nicholas	950	264	28	173	47	27	264	228	86	44
Limbé	425	109	26	77	29	38	109	65	60	43
Cap Haitien	1,956	413	21	365	111	30	413	303	73	39
Borgne	540	101	19	109	44	40	101	83	82	48
Saltrou	380	68	18	71	6	9	68	33	49	16
Grande Riviere	1,166	187	16	235	86	37	187	159	85	44
Port de Paix	1,020	119	12	213	38	18	119	98	82	25
Jacmel	1,934	222	12	389	56	14	222	147	66	20
Saint Marc	1,282	142	11	278	52	19	142	107	75	25
Grand'Anse	1,900	190	10	381	38	10	190	110	58	15
Marmelade	488	44	9	105	15	14	44	18	41	17
Gonaives	2,079	119	6	470	28	6	119	76	64	9
Vallieres	460	25	5	103	16	16	25	17	68	18
Dessalines	927	50	5	213	24	11	50	28	56	14
Port-au-Prince	5,519	285	5	1,289	112	9	285	109	38	10
Plaisance	574	8	1	132	12	9	8	3	37	9
Total	31,285	5,769	18	6,072	1,276	21	5,769	4,223	73	31

Sutter and Zuniga (11) it was found—as in the present survey—that the highest blood rate was in the No. IV group, but in their case only amounted to 29 per cent.

The species of malaria parasite encountered and their distribution by geographic areas is given in Table 8. It is striking that 86.6 per cent of all infections were due

to the *P. falciparum*. The next most frequent parasite found was *P. malariae* with 8.9 per cent. *Plasmodium vivax* accounted for only 1.9 per cent of the positive slides. The relatively small number of mixed infections encountered, 2.6 per cent, is partly to be explained by the great preponderance of the malignant tertian par-

TABLE 7

Association of Positive Parasite Findings with Degree of Splenic Enlargement—School Children of Haiti

ARRONDISSEMENT	CHILDREN WITH NO SPLEEN ENLARGEMENT		CHILDREN WITH SPLEEN P.D.I.		CHILDREN WITH SPLEEN I		CHILDREN WITH SPLEEN II		CHILDREN WITH SPLEEN III		CHILDREN WITH SPLEEN IV	
	No.	Per cent with parasites	No.	Per cent with parasites	No.	Per cent with parasites	No.	Per cent with parasites	No.	Per cent with parasites	No.	Per cent with parasites
Dessalines	213	11	39	46	9	89	2	100	0		0	
Gonaives	470	6	80	56	26	81	11	73	2	100	0	
Hinche	105	55	126	67	123	83	81	94	14	86	5	100
Saint Marc	278	19	101	71	26	85	15	93	0		0	
Marmelade	105	14	31	34	7	57	6	50	0		0	
Borgne	109	40	70	81	23	78	6	100	2	100	0	
Cap Haitien	365	30	207	72	155	77	42	90	9	22	0	
Fort Liberté	116	32	93	70	107	75	49	76	4	100	1	100
Grande Riviere	235	37	95	77	64	91	25	100	1	100	2	100
Limbé	77	38	26	50	61	64	19	58	3	67	0	
Plaisance	132	9	7	29	1	100	0		0		0	
Trou	79	46	90	72	85	87	34	85	6	83	0	
Vallières	103	16	11	55	12	79	1	100	1	100	0	
Mole St. Nicolas	173	27	120	97	67	85	70	40	7	100	0	
Port de Paix	213	18	73	75	33	91	10	100	3	100	0	
Jacmel	389	14	125	55	52	81	40	83	4	100	1	100
Lascahobas	62	16	58	55	36	61	9	78	1	100	1	100
Léogane	246	48	220	78	150	87	89	94	24	79	3	100
Mirebalais	74	41	106	78	59	76	37	76	5	80	0	
Port-au-Prince	1,289	9	180	29	78	56	22	55	4	25	1	100
Saltrou	71	8	24	29	29	62	12	50	2	50	1	100
Aquin	121	40	165	78	131	81	76	84	12	92	2	100
Cayes	342	32	309	65	241	79	99	77	20	85	0	
Coteaux	133	35	129	65	105	61	45	67	11	100	0	
Grand'Anse	381	10	101	46	58	69	23	61	7	100	1	100
Nippes	115	42	144	74	72	83	56	88	25	72	0	
Tiburon	76	22	83	54	42	65	27	67	13	62	0	
Total	6,072	21	2,813	68	1,852	78	906	80	180	80	18	100

asite and partly by the fact that slides were not ordinarily studied more than five minutes. Subsequent study of certain batches of these slides has shown a small percentage reported as straight species infection to have been in fact double. Triple infections occurred in at least nine instances. Eleven slides showing young ring forms only could not be positively diagnosed and are reported as of undetermined species.

Attempts to demonstrate a correlation between the species of parasite and the relative degree of splenic enlargement have met with no success in the present study. The small total of non-falciparum infections does not lend itself to extensive statistical analysis.

TABLE 8

Species of Malaria Parasites by Geographic Areas—Haiti, 1940–42

ARRONDISSEMENT	SPLEEN RATE	TOTAL SMEARS POSITIVE	FALCIPARUM INFECTION	PER CENT POSITIVE SMEARS	MALARIAE INFECTION	PER CENT POSITIVE SMEARS	VIVAX INFECTION	PER CENT POSITIVE SMEARS	MIXED INFECTION	PER CENT POSITIVE SMEARS
	per cent									
Aquin	43	364	323	88.7	17	4.7	6	1.6	18	4.9
Hinche	43	338	284	84.0	27	8.0	7	2.1	20	5.9
Mirebalais	40	190	147	77.4	32	16.8	9	4.7	2	1.1
Trou	38	218	191	87.6	11	5.1	13	6.0	3	1.4
Nippes	37	279	217	77.8	38	13.6	4	1.4	20	7.2
Tiburon	34	117	76	65.0	41	35.0	0	0.0	0	0.0
Coteaux	34	274	244	89.1	18	6.6	5	1.8	7	2.6
Fort Liberté	34	225	185	82.2	26	11.6	10	4.4	4	1.8
Léogane	32	519	465	89.6	36	6.9	5	1.0	13	2.5
Cayes	31	600	523	87.2	37	6.2	6	1.0	34	5.7
Lascahobas	28	73	62	84.9	9	12.3	2	2.7	0	0.0
Mole St. Nicolas	28	295	252	85.4	28	9.5	2	0.7	13	4.4
Limbé	26	94	77	81.9	15	16.0	2	2.1	0	0.0
Cap Haitien	21	414	380	91.8	29	7.0	3	0.7	2	0.5
Borgne	19	127	114	89.8	11	8.7	1	0.8	1	0.8
Saltrou	18	38	33	86.8	5	13.2	0	0.0	0	0.0
Grande Riviere	16	245	219	89.4	22	9.0	2	0.8	2	0.8
Port de Paix	12	135	126	93.3	9	6.7	0	0.0	0	0.0
Jacmel	12	203	186	91.6	11	5.4	5	2.5	1	0.5
Saint Marc	11	159	135	84.9	20	12.6	2	1.3	2	1.3
Grand'Anse	10	148	127	85.8	18	12.2	3	2.0	0	0.0
Marmelade	9	33	27	81.8	4	12.1	2	6.1	0	0.0
Gonaives	6	104	89	85.6	10	9.6	3	2.9	2	1.9
Vallieres	5	33	30	90.9	2	6.1	1	3.0	0	0.0
Dessalines	5	52	40	76.9	7	13.5	5	9.6	0	0.0
Port-au-Prince	5	215	201	93.5	9	4.2	4	1.9	1	0.5
Plaisance	1	15	15	100.0	0	0.0	0	0.0	0	0.0
Total		5,507*	4,768	86.6	492	8.9	102	1.9	145	2.6

* Eleven positive smears of undetermined species not tabulated.

ANOPHELINE SURVEY

No attempt was made in the present reconnaissance to attempt more than a brief search of the immediate vicinity of each school or village for anopheline breeding. In the vast majority of instances the visits were made during the long dry season or as soon as the trails were open following heavy rains. They were therefore often made at inopportune times. For this reason, failure to record the presence of the

TABLE 9

Species of Anophelines Collected by Departement, Arrondissement, and Commune

DEPARTEMENT	ARRONDISSEMENT	COMMUNE	NUMBER OF COLLECTIONS		
			Albimanus	Grabhami	Unidentified
Artibonite	Dessalines	Petite Riviere	6		
	Goraives	Ennery			1
		Gonaives	7	1	
		Gros Morne	9		6
	Hinche	Cerca Carvajal	4	7	
		Hinche	12	1	
		Maissade	16	1	
		Thomonde	4		1
	St. Marc	St. Marc	18	1	
Nord	Borgne	Port Margot	19	1	
	Cap Hatian	Acul du Nord	10	3	
		Limonade	5	1	
		Milot		5	3
		Plaine du Nord	9	3	
	Fort Liberté	Acul Samedi		10	
		Fort Liberté	4	3	1
		Grand Bassin	8	2	
		Ouanaminthe	5	5	
		Les Perches	7		
	Grand Riviere	Dondon	4	1	
		Grand Riviere	16		3
		St. Raphael	3		1
	Limbé	Limbé	2	4	
	Trou	Caracol	10		
		Terrier Rouge	4		
		Trou	2	2	
Nord-Ouest	M. St. Nicholas	M. St. Nicholas	7		
	Port-de-Paix	Port-de-Paix	4	5	
		St. Louis	4	5	
Ouest	Jacmel	Bainet	8	2	
		Jacmel	57	3	
		Marigot	10		
		Trouin	6	5	
	Las Cahobas	Belladere	4		
		Las Cahobas			3
	Léogane	Grand Goave	11	1	3
		Léogane	10		
		Petit-Goave	20		*
	Mirebalais	Sant d'Eau	8	6	
	Port-au-Prince	Ganthier	7		3
		La Gonave	6		
		Port-au-Prince	10		
	Saltrou	Saltrou	6		

TABLE 9—*Continued*

DEPARTEMENT	ARRONDISSEMENT	COMMUNE	NUMBER OF COLLECTIONS		
			Albimanus	Grabhami	Unidentified
Sud	Aquin	Aquin	15	2	
		Cavaillon	8		
		St. Louis du Sud	15		
	Cayes	Cayes	108	12	3
		Port-Salut	9	11	
		St. Jean du Sud	21	1	2
		Torbeck	24		
	Coteaux	Chardennieres	3		
		Coteaux	11		
		Roche a Bateau	5		
	Grand'Anse	Abricots	7		
		Corail	10	6	
		Moron	4	2	
		Roseaux	5		
		Trou-Bonbon	12		
	Nippes	Anse-a-Veau	6		
		Ansile	7		
		Baraderes	7	1	
		Grand Boucan	5		
		Petit Trou	10		
	Tiburon	Anse d'Hainault	10		
		Dame Marie	8		
		Les Irois	16		2
		Tiburon	4		2
Totals			764	113	31

* *A. Vestitipennis* identified.

vector does not exclude the possibility that it is locally abundant at some time of the year. Attempts to capture adults in the typical Haitian house were usually unsuccessful and were early abandoned to accelerate the pace of the reconnaissance.

Larvae were routinely placed in preservative and sent to the central laboratory for identification. In cases where only the young stages were found, they were sent in gauze stoppered bottles and kept alive for further development. This was particularly difficult in many cases since travel was often done by mule on precipitous trails and many lots were lost.

Three species of anophelines were found during the present survey, one of which is reported for the first time. These are *A. albimanus*, *A. grabhami*, and *A. vestitipennis*. Table 9 and Figure 4 indicate the distribution of these species with no attempt being made to represent relative abundance. Each symbol simply records the presence of the species in one or many water deposits in a locality.

Anopheles albimanus is almost universally present in Haiti. We identified it from collections from all arrondissements except three; namely, Marmelade, Plaisance, and Vallieres. These were mountainous regions well removed from the coast

FIG. 4. LOCALITIES WHERE ANOPHELINE LARVAE WERE FOUND IN ONE OR MANY WATER DEPOSITS. NO ATTEMPT IS MADE TO SHOW RELATIVE ABUNDANCE

with elevations ranging from 1,020 to 3,500 feet. The second species with a wide distribution was *A. grabhami*. It was found in 19 of the 27 arrondissements. The third species, *A. vestitipennis*, was identified on several occasions from routine collections made at Petit-Goave, where control work was being initiated. It was found in a shaded swamp area near an old coffee-washing plant which was being used for making concrete ditch linings.

Anopheles albimanus was found to have a wide variety of breeding places but usually preferred a sunlit situation. Specimens were recovered from clear springs and from polluted puddles, cool seepage outcrops and hot roadside ditches, rice fields and brackish mangrove thickets, hoofprints, and large lakes. It bred throughout the year at lower elevations but was most abundant immediately following the rainy season.

Anopheles grabhami was often found to be associated with *albimanus* in the same water deposit but would be found alone in streams and canals that were heavily shaded. They were relatively more abundant in the winter or dry season. *A. grabhami* was the only species encountered in the communes of Milot and Acul Samedi, two places where malaria transmission is known to occur. We were denied an opportunity definitely to determine whether or not this species alone is the local vector. Up to the present, it has not been considered of practical importance in any part of the West Indies.

DISCUSSION

The observations made during the course of this reconnaissance on the distribution of *Anopheles albimanus* and the data collected on the incidence of malaria in the various sections of the country have made it possible for the national health authorities to initiate a planned program of malaria abatement. The detailed data on individual schools, which is not presented in this paper, upon further analysis should direct the attention of local field workers to many small but important control projects which could be carried out with a minimum financial outlay. Needless to say, numerous dangerous breeding areas are man-made, for example, those arising from improper practices in road building, uncontrolled irrigation, undrained borrow pits, and the like. Closer cooperation in sanitary matters between the various government departments and important agricultural interests should be able to prevent this in the future.

Spleen and parasite rates presented for many areas of Haiti are as high, or higher, than those reported from anywhere in the Western Hemisphere. Nevertheless, the clinical manifestations of malaria rarely arrest attention except among the infants of the community. To a large extent this can be explained by a reasonably high tolerance to the disease due to repeated attacks in early life. But it is well to remark that the population of the island, being very largely of African descent, enjoys the relative racial immunity often noted in members of the negro race. In fact, the situation in Haiti is remarkably like that reported from Sierra Leone by Macdonald (12) and from the Lower Congo by Schwetz and Geronnez (13).

Another striking similarity between malaria in the Haitian population and that of the West Coast of Africa is the preponderance of infections by *P. falciparum* and the virtual absence of *P. vivax*. In Haiti, as in Africa, *P. malariae* cases are

irregularly distributed and rarely amount to more than a small fraction of the total. Owing to the fact that our records are based on a single visit to each community we are unable to determine the relationship between season and the species of parasite encountered. In some surveys the relative incidence of the three species varied considerably at different times of the year in the same population group.

SUMMARY AND CONCLUSIONS

1. A total of 31,285 spleen palpations were made in 826 schools of Haiti. Splenic enlargement was noted in 18.4 per cent of the children.

2. The most malarious areas are the arrondissements of Aquin, Hinche, Mirebalais, Trou, Nippes, Tiburon, Coteaux, Fort Liberté, Leogane, and Cayes—all with spleen rates higher than 30 per cent. The few areas of Haiti that are malaria free, are the higher mountain valleys and the southwest slopes where the rainfall is very low.

3. Blood smears were made on 11,841 children, and the parasite rate, adjusted to all those given spleen examinations, was 31 per cent.

4. The most common type of parasite was found to be *P. falciparum*, which was responsible for 86.6 per cent of the total infections; *P. malariae* accounted for 8.9 per cent, and *P. vivax* for only 1.9 per cent.

5. Three species of *Anopheles* were found in Haiti. *A. albimanus*, the important local vector, was present in 24 out of 27 arrondissements. *A. grabhami* was also widely distributed, being found in 19 arrondissements. *A. vestitipennis* was discovered in only one locality and is reported for the first time.

RESUMEN Y CONCLUSIONES

1.—Se palparon 31.285 bazos en 826 escuelas de Haití. Se encontró un agrandamiento esplénico en 18.4 por ciento de los niños.

2.—Las áreas más maláricas son los alrededores de Aquin, Hinche, Mirebalais, Trou, Nippes, Tiburon, Coteaux, Fort Liberte, Leogane, y Cayes—todas con rata esplénica más alta del 30 por ciento. Las pocas áreas de Haití que están libres de Malaria son los altosvalles montañosos y las vertientes del suroeste donde la precipitación de lluvia es muy baja.

3.—Se examinaron gotas gruesas de sangre de 11.841 niños y el índice parasitario, ajustado a todos aquellos que dieron exámenes de bazos fué del 31 por ciento.

4.—El tipo más común de parásito encontrado fué el *P. falciparum* que fué responsable del 86.6 por ciento de las infecciones totales; el *P. malariae* fué el 8.9 por ciento y el *P. vivax* únicamente el 1.9 por ciento.

5.—Tres especies de *Anopheles* fueron encontradas en Haiti. *Anopheles albimanus*, el vector local importante estuvo presente en 24 de los 27 sitios examinados. *A grabhami* fué también encontrado ampliamente distribuído en las áreas en 19 de los sitios donde se buscó. *A. vestitipennis* fué descubierto únicamente en una localidad y es reportado por la primera vez en Haití.

ACKNOWLEDGMENTS

We should like to express our gratitude to the many officials of various government departments who actively aided the survey, particularly, to the former Director of

the Service d'Hygiene, Dr. Rulx Leon, upon whose initiative the study was undertaken. His successors, Dr. Louis Hippolyte and Dr. Jules Thébaud have also given every possible encouragement to the organization of the present Malaria Control Service which developed as an outgrowth of the reconnaissance.

The late Dr. P. J. Crawford, who was director for the Caribbean Region for the International Health Division of The Rockefeller Foundation, and Dr. Mark F. Boyd, Director of the Station For Malaria Research, Tallahassee, Florida, and member of the staff of International Health Division of The Rockefeller Foundation, gave valuable advice and assistance throughout the study.

Special mention should be made of the untiring service of Drs. Charles Dambreville, Morice Hall, and Charles Fontus, all of whom were at one time members of the field units.

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STANDARDIZATION OF A TEST FOR ANTIMALARIAL EFFECT WITH PLASMODIUM CATHEMERIUM IN THE CANARY[1]

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For determination of the minimum therapeutic dose of compounds with blood induced infection we have used a strain of *Plasmodium cathemerium* derived from the 3H2 strain (Beckman 1942) by repeated blood transfers in canaries over a long period of time. This strain[2] is designated as 3H2-1[3] and is characterized by a relatively mild infection and very low fatality rate (less than 1%). No exo-erythrocytic forms have been found in repeated examinations nor have we observed any splenic infarction, pericarditis, myocarditis, thrombosis of vessels of the brain, etc.

The course of infection following intravenous inoculation of various numbers of parasites was studied. The number of parasites for a given inoculum was determined by counting the number of parasites per 1000 erythrocytes in a blood film of the intended donor, counting the number of erythrocytes per cu mm of blood and calculating the number of parasites per unit volume. In the experiment reported here the six different inocula ($5 \times$, $2 \times$, $1 \times$, $0.5 \times$, $0.25 \times$ and 0.1×10^6 parasites) were all prepared by proper dilutions of the same pool of infected blood, using 5 per cent normal canary blood in physiological saline as the diluent. Each inoculum was injected intravenously in 0.2 ml into each of 10 birds (selected for 15 to 20 grams). An additional 5 birds each received 0.5×10^6 parasites, and quinine dihydrochloride 0.147 mg, in 0.2 ml by stomach tube twice per day for 4.5 days, beginning soon after inoculation, i.e., the afternoon of day 0. Blood films were made in the morning daily and the number of parasites counted per unit number of erythrocytes. The size of sample counted was never less than 1000 erythrocytes and always sufficient for a probable error of 10 per cent or less (Gingrich 1932).

The daily average numbers of parasites per 1000 erythrocytes for each group are plotted semi-logarithmically in Figure. 1. With an inoculation of one-half million parasites the peak of infection occurs regularly on the fifth day after inoculation. This permits treatment for 4.5 days between the time of inoculation and the time of determination of effect. During this time there occurs a steady increase in the number of parasites each day when the inoculum contains 0.5×10^6 parasites. Following inoculation of 0.25×10^6 parasites a portion of the infections do not reach

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Texas. The authors acknowledge the assistance of Mrs. Charles H. Cox, Jr., Mrs. Harold G. Sleeper, Jr., Catherine C. Shepherd, Sarah B. Lyon, Marjorie Schwab and Cora Alice Taylor.

[2] Obtained from Dr. Clay G. Huff.

[3] By reference to the Committee on Terminology of Strains of Avian Malaria of the American Society of Parasitologists.

their peak by the fifth day but such infections are useful because there is regular daily multiplication of the parasites. When 0.1×10^6 parasites are inoculated, however, the height of infection becomes too irregular in both magnitude and in occurrence to be serviceable for accurate chemotherapeutic testing. On the other hand, when more than one-half million parasites are inoculated the difficulty arises from the interference of acquired immunity. By the time an adequate period of time for treatment has elapsed many of the infections are well on their way to recovery. With inoculation of 1×10^6 parasites a few infections are barely past their peak and with $2 \times$ and 5×10^6 most of the infections have reached a peak by the fourth or third days after inoculation. It is evident, therefore, that the optimum size of inoculum for infections with a 4- to 5-day treatment period is 0.5×10^6 and the limits of technical error for useful infections are from half to double that number.

Depending on the objective desired, the type of therapeutic test may be varied according to the dosage and administration of drug. Of the various effects to be determined there are two which deserve mention: (1) the amount of drug required to produce effect equivalent to a given amount of quinine (expressed as a ratio and commonly called the "quinine equivalent"), which is virtually the minimum therapeutic dose; and (2) the degree of suppression, or cure, with maximum tolerated doses. The minimum therapeutic dose of quinine base in many experiments (with inoculation of 0.5×10^6 parasites of strain 3H2-1) has usually been 0.24 mg per day. This usually reduced the parasitemia to one-third or one-fourth of that in controls on the fifth day.

In the experiment described above the means and standard deviations of the fifth day observations of the 10 birds inoculated with 0.5×10^6 parasites and without drug, and the 5 birds with the same number of parasites and treated with quinine dihydrochloride 0.147 mg twice per day (0.24 mg quinine base per day) are represented in Figure 2. The parasitemia of the treated birds is approximately one-third that of controls and the standard deviations do not overlap. In practice, blood examinations are made on the fourth day as well as on the fifth to guard against possible error in the event that the peak of control infections might occur earlier than anticipated. This latitude for determining effectiveness or ineffectiveness with a given dose of a compound is very small as compared with other strains, 3C and 3T (Huff, Boyd and Manwell 1944 and Wolfson 1940), of *Plasmodium cathemerium* and as compared with the same species and *P. lophurae* in young ducks (Hewitt 1942 and Marshall[4]) and *P. lophurae* and *P. gallinaceum* in chicks (Porter[4] and Coatney[4]).

To illustrate the limits for determining the minimum effective dose of quinine, for example, we have plotted the results obtained with fixed doses over a period of two years in Figure 3. The number of observations for the several points varies from 175 for controls to relatively few for 11.4 and 22.9 mg/Kg-day. Doses of 9.1, 13.7 and 18.3 mg/Kg-day were regularly used for each test. It should be mentioned that these figures represent the total daily dose of quinine base and are obtained by assuming the average weight of 17.5 grams whereas the canaries were merely selected for 15 to 20 grams. Furthermore, the intervals between doses are

[4] Personal communication.

adjusted rather closely to the range in bird weight. Thus, 0.098 mg quinine dihydrochloride twice per day for a 20-gram bird is equivalent to 0.13 mg. twice per day for a 15-gram bird (or 9.1 mg. quinine base per Kg-day for an average 17.5-gram bird); 0.147 mg. quinine dihydrochloride twice per day for a 20-gram bird is equivalent to 0.196 mg. twice per day for a 15-gram bird (or 13.7 mg. quinine base per Kg-day for an average 17.5 bird); 0.196 mg quinine dihydrochloride twice per day

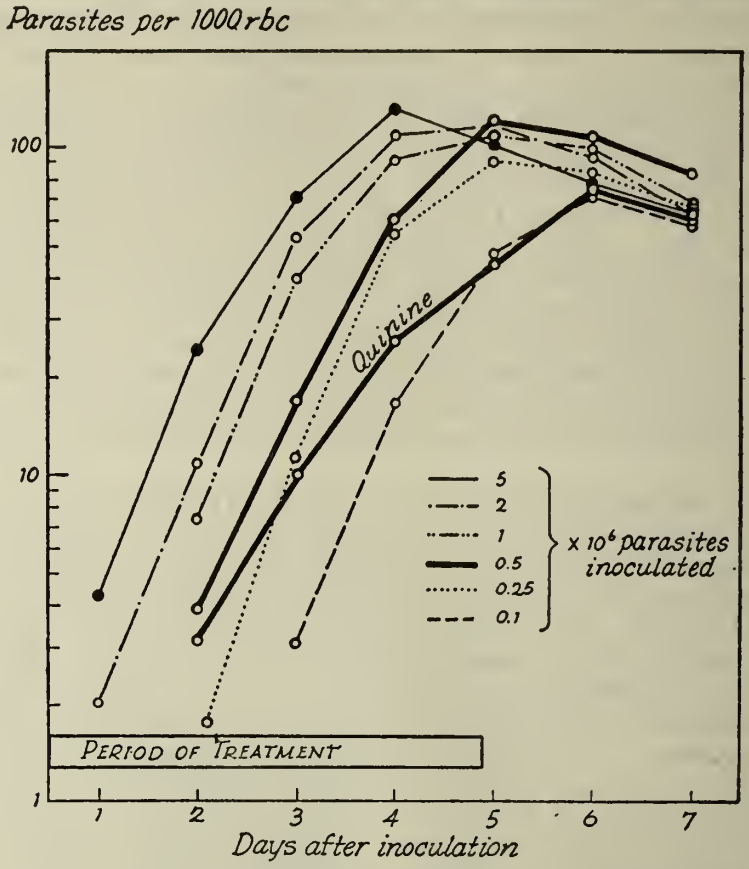

Fig. 1. Course of infection in canaries with strain 3H2-1 of *Plasmodium cathemerium* following intravenous inoculation of various numbers of parasites; and with 0.147 mg. quinine twice per day for 4.5 days following inoculation of 0.5×10^6 parasites.

for a 20-gram bird is equivalent to 0.261 mg twice per day for a 15-gram bird (or 18.3 mg quinine base per Kg-day for an average 17.5-gram bird); and 0.245 mg quinine dihydrochloride twice per day for a 20-gram bird is equivalent to 0.327 mg twice per day for a 15-gram bird (or 22.9 mg quinine base per Kg-day for an average 17.5-gram bird). The range of dose when 0.147 mg quinine dihydrochloride is administered twice per day to canaries selected for 15 to 20 grams is 12 to 16 mg quinine base per Kg-day. In our experience similarly narrow limits for determining

minimum effective doses applied to many compounds in variable degree when tested with strain 3H2-1 in canaries.

As an example of determining the quinine equivalent of a given compound we may consider the data available in one experiment with Pamaquine (Plasmochin) naphthoate and Pamaquine iodide. The procedure of experiment was as described above with intravenous inoculation of 0.5×10^6 parasites and treatment for 4.5 days, using four birds for each group. The data are given in Table 1, together with the calculations for the quinine equivalents. The points of reference for the

FIG. 2. Means and standard deviations of untreated and treated birds (quinine dihydrochloride 0.147 mg. twice per day for 4.5 days) as observed on the fifth day after inoculation of 0.5×10^6 parasites.

calculations are the minimum effective doses with nearly equivalent parasitemias on the fifth day (0.24 quinine base, 0.01067 mg pamaquine naphthoate, 0.0072 mg pamaquine iodide and 0.0048 mg pamaquine base). The question may arise why 0.32 mg quinine base is not used in reference to 0.01067 mg pamaquine naphthoate since the parasitemias are more closely approximate.[5] After considerable experience it was found advisable to use 0.24 mg quinine as the reference point regularly except in rare instances when it was ineffective. With the relatively small number

[5] This would yield a quinine equivalent of 30. The ratio of 0.24 (quinine base) and 0.008 (pamaquine naphthoate) is also 30 but their parasitemias are far from equivalent.

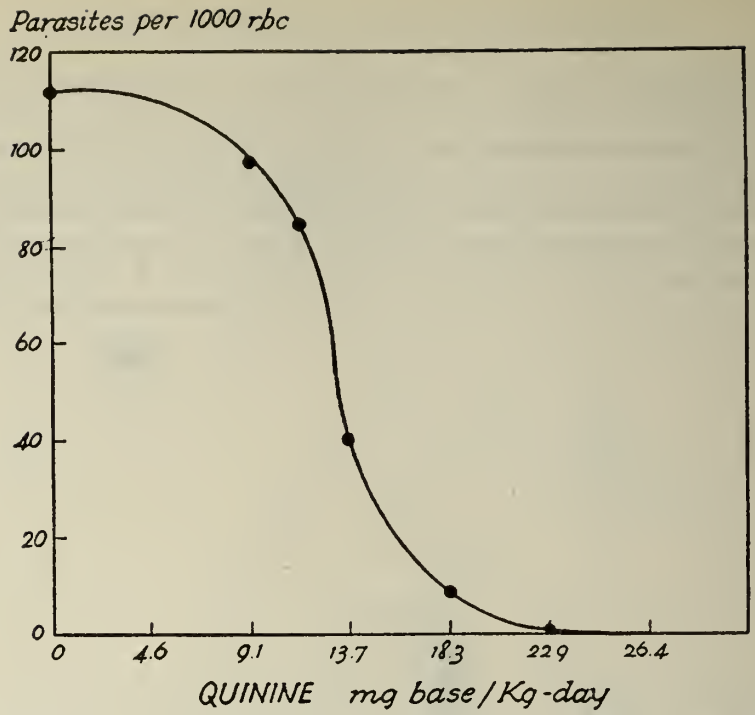

FIG. 3. The effect of increasing doses of quinine on the parasitemia of the fifth day after inoculation of 0.5×10^6 parasites.

TABLE 1

Determination of Minimum Effective Dose (Quinine Equivalent) of Pamaquine naphthoate and Pamaquine iodide

DRUG AND DAILY DOSE	DRUG BASE EQUIVALENTS	PARASITEMIA 5TH DAY
mg.	*mg.*	
Controls		97.25
Quinine dihydrochloride		
0.196	0.16	89.5
0.294	0.24	34.75
0.392	0.32	9.0
Pamaquine naphthoate		
0.00534	0.0024	87.25
0.008	0.0036	79.75
0.01067	0.0048	16.25
0.01333	0.006	3.75
Pamaquine iodide		
0.0036	0.0024	87.5
0.0054	0.0036	54.0
0.0072	0.0048	25.75
0.009	0.006	5.75

Quinine equivalent of:

Pamaquine naphthoate (44.8% base) = 0.24 ÷ 0.01067 = 23.43.

Pamaquine iodide (66.15% base) = 0.24 ÷ 0.0072 = 33.33.

Pamaquine base = 0.24 ÷ 0.0048 = 50.

of animals (four) in each group the average parasitemia with this dose of quinine has run as low as 2. To change the quinine reference point for each variation in average parasitemia would introduce this variable as well as the similar variable with the compound under study in the calculations of quinine equivalents. It is also significant that a parasitemia of 16.25 falls within the lower limit of the standard deviation of the quinine reference point of 0.24 mg.

By way of discussion it may be mentioned that there is thus far no evidence, neither direct nor indirect, for the occurrence of exo-erythrocytic forms in strain 3H2-1 of *P. cathemerium* in the canary. It is therefore considered useful for therapeutic tests indicating effect exclusively on erythrocytic parasites. This consideration as well as the value of this particular strain for curative tests[6] justify the use of strain 3H2-1 for use in routine therapeutic testing in spite of the narrow limits for determining quinine equivalents. The latter feature is disadvantageous only in requiring more tests to reach an end point and more tedious counting for low parasitemias. In fact, it yields more accurate determinations of quinine equivalents. It may be accounted for by the relative avirulence of the strain as compared with 3C and 3T and probably also by the fact that the hosts are adults, for *P. lophurae*, for example, produces severer infections in chicks and ducklings than in grown animals (Coggeshall 1938 and Hewitt 1942). One simple advantage of using canaries is that less of the test drug is required. The administration of drug by stomach tube is time-consuming and discontinuous as compared with the drug diet method.

SUMMARY

1. Standardization of a therapeutic test for antimalarial effect (exclusively for effect on erythrocytic parasites) with *Plasmodium cathemerium* in the canary is described.

2. The variation occurring in practice with the test as well as the advantages and disadvantages are discussed.

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STUDIES ON SEROLOGY OF MALARIA

III. Malarial Precipitation Reactions with Lipid Antigen[1]

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A preliminary report is herewith presented of studies on serologic reactivity of malarial sera with lipid antigen. It is well known that certain malarial sera give serologic reactions with lipid antigens employed in tests for syphilis. The question arose, whether, by increasing the nonsyphilitic sensitivity of these tests through modifications of the antigen and technique, the number of positive reactions with malarial sera would be distinctly greater than with nonmalarial sera. It was believed that these studies would help answer the question whether malarial sera possess a definite tendency toward serologic reactivity with lipid antigen.

Several factors which increase nonsyphilitic serologic reactivity with lipid antigen were utilized. These factors were observed in this laboratory in connection with studies on the verification test in the detection of "false positive" serologic reactions (1). The factors include: (1) reduction in NaCl concentration in the preparation of the antigen-salt solution suspension employed in the tests; (2) use of excessively sensitive antigen; (3) use of water (instead of physiologic salt solution) in preparing serial dilutions of serum, and (4) incubation of serum-antigen mixtures at icebox temperature. The results, as will be seen below, indicate that malarial sera possess considerably more serologic reactivity with lipid antigens than nonmalarial sera and efforts will be directed in future studies toward still greater differentiation between malarial and nonmalarial sera.

Differentiation between malarial and nonmalarial sera by means of precipitation tecnhiques were reported by Taliaferro et al. (1927, 1928), Henry (1927), Row (1946), Dulaney (1941) and Wolff (1939). The complement fixation studies reported by Coggeshall and Eaton (1938, 1939) led to many investigations into the applicability of this phenomenon to the diagnosis of malaria. Dulaney and her coworkers (1940, 1942, 1944) have been active contributors in this field, and more recently, Rein and Bucholtz (1946) have studied this phenomenon. In 1938 Eaton (1938) reported an agglutination test for the diagnosis of malaria, but no further reports have been made on this technique. Sinton and Mulligan (1932) reported cutaneous responses to injected malarial antigen in infected persons. Dulaney and Stratman-Thomas failed to confirm these findings. A biochemical test in the diagnosis of malaria was developed by Proske and Watson (1939), but its value and limitations have not as yet been fully investigated.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Regents of the University of Michigan.

MATERIALS AND METHODS

Three antigens were used in this study: Standard Kahn antigen, sensitized antigen and excessively sensitive antigen. (1) *Standard antigen.* This is the antigen used in the standard Kahn test and its preparation and standardization are described elsewhere (Kahn, 1946). (2) *Sensitized antigen.* This antigen is used in the presumptive Kahn test which is about 10 per cent more sensitive than the standard test in known cases of syphilis, but is not quite as specific as the standard test. The preparation and standardization of sensitized antigen are described elsewhere. (3) *Excessively sensitive antigen.* This antigen when employed in the Kahn test in place of standard Kahn antigen gives about 20 per cent of positive reactions with nonsyphilitic sera. Powdered beef heart which had aged for periods from several months to more than a year were used in the preparation of this antigen. The method or preparation and standardization of this antigen was carried out according to the techniques of standard Kahn antigen.

TABLE 1

Titers of Lipid Antigens

The titers: 1 cc. antigen plus amount of salt solution of a given concentration

	CONCENTRATION OF SODIUM CHLORIDE SOLUTIONS			
	0%	0.3%	0.6%	0.9%
Standard antigen				
Antigen #1	1 + 0.75	1 + 1.15	1 + 1.1	1 + 1.2
Sensitized antigen				
Antigen #1	1 + 0.65	1 + 1.3	1 + 1.6	1 + 2.0
Excessively sensitive antigen				
Antigen #1	1 + 0.65	1 + 1.2	1 + 1.5	1 + 2.15*
Antigen #2	1 + 0.7	1 + 1.2	1 + 1.7	1 + 2.25
Antigen #3	1 + 0.6	1 + 1.1	1 + 1.6	1 + 1.95
Antigen #4	1 + 0.7	1 + 1.2	1 + 1.5	1 + 2.05

* The titers of all excessively sensitive antigens as determined with 0.9% or more salt solutions were not usable because of instability of the final suspensions.

Antigen Titrations. Each of the three antigens were titrated with water, with 0.3 per cent NaCl solution, with 0.6 per cent and with 0.9 per cent salt solution, respectively. Table 1 gives the titers of these antigens with the several concentrations of NaCl solution. In the case of standard antigen, the titers were found to be 1 cc. antigen plus 0.75 cc. water; 1 cc. antigen plus 1.15 cc. of 0.3 per cent NaCl solution; 1 cc. antigen plus 1.1 cc. of 0.6 per cent NaCl solution and 1 cc. antigen plus 1.2 cc. of 0.9 per cent NaCl solution. Sensitized and especially excessively sensitive antigens required somewhat larger amounts of the salt solutions for their titers than standard antigen.

Sera employed in the tests were separated from the clot in the usual manner and were heated for 30 minutes at 56°C. before using.

Performance of Tests. The antigen suspensions were prepared in the usual manner by mixing 1 cc. of the antigen with an amount of salt solution (or water) indicated by the titer. The suspensions were aged for 10 minutes before using and were not employed when aged more than 30 minutes. Preliminary experiments indicated the desirability of employing in the tests two types of antigen suspensions; one prepared with 0.9 per cent NaCl solution (referred to in the text as "0.9 per cent system") and the other prepared with 0.3 per cent NaCl solution (referred to as "0.3 per cent system").

The use of various ratios of serum:antigen suspension were at first undertaken. In addition to the 3:1, 6:1 and 12:1 ratios of serum to antigen suspension employed in the Kahn test, other ratios, such as, 18:1, 24:1 and 36:1 were also employed. Preliminary experiments indicated that 6:1, 12:1 and 24:1 were the ratios to be preferred. Micro amounts of serum and antigen suspension were employed. Generally, 0.01 cc. of antigen suspension was employed with the appropriate amount of serum to bring about the desired ratio of serum to antigen suspension.

The antigen suspension and serum were measured into Kahn tubes and, after mixing, the rack was shaken for three minutes in the shaking machine. To each tube was then added 0.5 cc. of diluent. This diluent was of the same NaCl concentration as that used in preparing the antigen suspension. Thus, if 0.3 per cent NaCl solution was used in preparing the antigen suspension, 0.3 per cent solution was used as diluent after the shaking period. If 0.9 per cent NaCl solution was used in preparing the antigen suspension, the same concentration of salt solution was used as diluent. The results were read after the addition of diluent to the tubes and shaking for about ten seconds to mix the ingredients. Other readings were made after incubation periods at 37°C. and at icebox (1° to 10°C.) temperature. It was soon observed that incubation at 37°C. led almost entirly to negative results. Hence, cold temperature incubation was resorted to almost entirely.

EXPERIMENTAL

Comparative Precipitation Results with Malarial and Nonmalarial Sera Employing Various Antigens

The nonmalarial sera were obtained from the serology laboratory of the University of Michigan Hospital after they had been examined with the Kahn test. Malarial blood specimens were kindly sent to us by the U. S. Naval Medical Center, Bethesda, and by the San Diego Naval Hospital, San Diego, California. The sera were prepared in the usual manner and were heated for 30 minutes at 56°C. before using. Three antigens were employed: standard Kahn antigen, sensitized antigen, and excessively sensitive antigen. Preliminary experiments indicated that, in the preparation of antigen suspensions, the use of 0.3 per cent NaCl solution (0.3 per cent NaCl system) and of 0.9 per cent NaCl solution (0.9 per cent NaCl system) were most desirable. This applied to standard antigen as well as to sensitized and excessively sensitive antigens. A ratio of serum: antigen suspension of 6:1 was employed in this experiment. The amount of serum used was 0.15 cc. and the amount of antigen suspension was 0.025 cc. The serum: antigen mixture was shaken for three minutes in the Kahn shaker. After shaking, 0.5 cc. of salt solution of the same concentration used in preparing the antigen suspension was added to each tube. The tests were taken for 10 seconds by hand to permit thorough mixing and the precipitation reactions read. Additional readings were made after 1, 4, and 20 hours incubation in the icebox.

Table 2 gives illustrative precipitation results obtained with malarial and nonmalarial sera employing the three antigens. With standard antigen, malarial sera showed a greater tendency toward precipitation than nonmalarial sera both, in the 0.3 per cent and the 0.9 per cent NaCl systems. With sensitized antigen the difference between malarial and nonmalarial sera was not as marked. With excessively sensitive antigen in the 0.3 per cent system malarial sera also showed a greater tendency toward precipitation than nonmalarial sera. The tests with standard and sensitized antigens were incubated for four hours at icebox temperature and the

tests with excessively sensitive antigen for one hour at the same temperature. Prolonged incubation in the cold tended to render practically all tests positive.

TABLE 2

Illustrative Precipitation Results with Various Antigens Employing Malarial and Nonmalarial Sera

Ratio of serum:antigen suspension 6:1

SERUM NO.	STANDARD ANTIGEN*		SENSITIZED ANTIGEN*		EXCESSIVELY SENSITIVE ANTIGEN	
	Malarial sera	Nonmalarial sera	Malarial sera	Nonmalarial sera	Malarial sera	Nonmalarial sera
0.3% NaCl System						
1	4†	4	4	4	4	4
2	4	1	4	4	4	4
3	4	—	1	—	4	4
4	1	—	—	—	4	—
5	—	—	—	—	4	—
0.9% NaCl System						
6	4	2	4	4	0	0
7	4	—	4	—	0	0
8	—	—	—	—	0	0
9	—	—	—	—	0	0
10	—	—	—	—	0	0

* The tests with standard and sensitized antigen were incubated for 4 hours in the icebox before reading and the tests with excessively sensitive antigen for 1 hour in the icebox.

† 4, 3, 2, and 1 = degrees of precipation; — = negative reaction; 0 = no test made.

TABLE 3

Summary of Precipitation Results with Various Antigens, Employing Malarial and Nonmalarial Sera

STANDARD ANTIGEN*				SENSITIZED ANTIGEN*				EXCESSIVELY SENSITIVE ANTIGEN			
Malarial sera		Nonmalarial sera		Malarial sera		Nonmalarial sera		Malarial sera		Nonmalarial sera	
No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos-	No. tested	% pos.
0.3% NaCl System											
68	60	224	32	32	47	233	38	32	97	155	59
0.9% NaCl System											
47	38	190	5	24	37	88	12	0	0	0	0

* Standard and sensitized antigen tests were incubated for 4 hours in the icebox before reading and excessively sensitive antigen tests for 1 hour in the icebox.

Table 3 summarizes the precipitation results obtained with malarial and nonmalarial sera, employing standard and sensitized antigens in 0.3 per cent and 0.9 per cent NaCl systems, and excessively sensitive antigen in a 0.3 per cent system. Of

68 malarial sera tested with standard antigen and the 0.3 per cent sytem, 60 per cent gave positive reactions and of 224 monmalarial sera tested, 32 per cent gave positive reactions. Of 47 malarial sera tested with standard antigen and the 0.9 per cent system, 38 per cent gave positive reactions; of 190 nonmalarial sera tested, 5 per cent gave positive reactions.

Of 32 malarial sera tested with sensitized antigen and the 0.3 per cent system, 47 per cent gave positive reactions and of 233 nonmalarial sera, 38 per cent gave positive reactions. Of 24 malarial sera tested with sensitized antigen and the 0.9 per cent system, 37 per cent gave positive reactions and of 88 nonmalarial sera, 12 per cent gave positive reactions. Of 32 malarial sera tested with excessively sensitive antigen and the 0.3 per cent system, 97 per cent gave positive reactions and of 155 nonmalarial sera, 59 per cent gave positive reactions.

It is evident from these results that the highest degree of differentiation between malarial and nonmalarial sera occurred with standard antigen and the 0.9 per cent system; namely 38 per cent against 5 per cent. The greatest percentage of positive reactions with malarial sera, and to a lesser extent with nonmalarial sera, was obtained with excessively sensitive antigen and the 0.3 per cent system; namely, 97 per cent against 59 per cent.

Quantitative Precipitation Results with Malarial and Nonmalarial Sera Employing Standard Antigen

Experience in this laboratory has shown that the sensitivity of mixtures of nonsyphilitic sera and Kahn antigen suspension is increased by dilution of the serum with water, and it seemed of interest to find whether malarial sera would give precipitation reactions under these conditions. The following experiment was accordingly carried out by employing serial dilutions of malarial and nonmalarial sera with water and antigen suspension prepared with 0.3 per cent NaCl solution. The serum dilutions were 1:15, 1:30, 1:60, 1:120, 1:480, 1:960, and 1:1920 and these were employed with antigen suspension in ratios of 1:1, 3:1, 6:1, 12:1, 18:1, 24:1, and 30:1. The mixtures were shaken by hand for 10 seconds, to mix the ingredients, then for 3 minutes in the Kahn shaker. The tests were read immediately without the addition of diluent.

Preliminary experiments with malarial and nonmalarial sera indicated that the number of serum dilutions and ratios could be reduced without sacrificing a significant number of positive reactions. The dilutions chosen for further studies were thereupon limited to 1:15 through 1:240, and the ratios of serum: antigen suspension chosen were 6:1, 12:1, and 24:1. Table 4 gives illustrative precipitation results under these experimental conditions. It is evident that serial dilutions of malarial serum with water show a greater tendency toward precipitation with antigen suspension than nonmalarial sera.

Table 5 presents comparative precipitation results in the 0.3 per cent system with malarial and nonmalarial sera in various serial dilutions with water. One hundred and fifteen malarial sera and 155 nonmalarial sera were employed in this experiment. It is evident that 93, 87, and 81 per cent of the malarial sera showed precipitation in the 1:15 and 1:30 serum dilutions with water in ratios of serum: antigen

of 6:1, 12:1, and 24:1, respectively. The corresponding percentages of precipitation given by the nonmalarial sera under the same experimental conditions were 15, 10, and 7 per cent. It was believed, however, that the differences in the ages of the malarial and nonmalarial sera might have affected the above results. The malarial blood specimens were received by mail at intervals of 24 to 72 hours after collection

TABLE 4

Illustrative Precipitation Results Given by Malarial and Nonmalarial Sera on Dilution with Water and Mixing Dilutions with Antigen Suspension

DILUTIONS OF SERUM WITH WATER	MALARIAL SERUM			NONMALARIAL SERUM		
	Serum:antigen ratios					
	6:1	12:1	24:1	6:1	12:1	24:1
1:15	4	4	4	4	4	2
1:30	4	4	4	4	2	—
1:60	4	4	4	2	—	—
1:120	4	4	4	—	—	—
1:240	2	—	—	—	—	—

TABLE 5

Comparative Precipitation Results in 0.3% System with Malarial and Nonmalarial Sera in Various Dilutions with Water

DILUTIONS OF SERUM IN WHICH PRECIPITATION OCCURRED	115 MALARIAL SERA						155 NONMALARIAL SERA					
	Serum:Antigen Ratios											
	6:1		12:1		24:1		6:1		12:1		24:1	
	No. sera	% pos.*	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.
1:15 –1:240	90	78.3	90	78.3	90	78.3	14	9	13	8.4	11	7.1
1:30 –1:240	17	14.8	10	8.7	3	2.6	9	5.8	3	2.0	0	
1:60 –1:240	3	2.6	3	2.6	2	1.7	26	16.7	5	3.2	2	1.3
1:120–1:240	2	1.7	1	0.9	1	0.9	29	18.9	9	5.8	1	0.7
1:240	1	0.9	0		0		39	25	9	5.8	5	3.2
		% neg.		% neg.		% neg.		% neg.		% neg.		% neg.
Negative in all dilutions	2	1.7	11	9.6	19	16.5	38	24.4	116	74.8	136	87.7

* Precipitation of two plus or higher was considered a positive reaction.

while the nonmalarial specimens were obtained daily from the serology laboratory of the University Hospital and were generally not more than 24 hours old.

A similar experiment was thereupon carried out employing 48 fresh and 21 aged nonmalarial sera and 18 fresh malarial sera. The results of this experiment are presented in Table 6. The data in this table show that ageing of the nonmalarial sera for 24 to 48 hours at room temperature increased the tendency toward precipi-

tation. Thus, 39 per cent of the fresh sera began to show precipitation in the 1:15 and 1:30 serum dilutions with water and in a 6:1 ratio of serum to antigen while 62 per cent of the aged sera began to show precipitation under the same conditions. This increase in precipitation after ageing was not observed in the 12:1 and 24:1 ratios. In the case of malarial sera, ageing was also found to increase the tendency toward precipitation. These sera in 1:15 and 1:30 dilutions with water and in a serum: antigen ratio of 6:1 gave 67 per cent precipitation. Malarial sera aged under similar experimental conditions, as was already seen in Table 5, showed 93 per cent precipitation. In the 12:1 serum: antigen ratio, 67 per cent fresh malarial sera showed precipitation and in the 24:1 ratio, 28 percent showed precipitation. With aged malarial sera in the same ratios, precipitation was noted in 87 per cent and 81 per cent of the sera, respectively (Table 5).

TABLE 6

Comparative Precipitation Results in 0.3% System with Fresh and Aged Malarial and Nonmalarial Sera in Various Dilutions with Water

DILUTIONS OF SERUM IN WHICH PRECIPITATION OCCURRED	48 FRESH NONMALARIAL SERA						21 AGED NONMALARIAL SERA*						18 FRESH MALARIAL SERA					
	Serum:antigen Ratios																	
	6:1		12:1		24:1		6:1		12:1		24:1		6:1		12:1		24:1	
	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.	No. sera	% pos.
1:15 –1:240	14	29	16	33	8	17	6	29	4	19	1	6	10	56	10	56	5	28
1:30 –1:240	5	10	1	2	1	2	7	33	3	14	1	6	2	11	2	11	0	
1.60 –1:240	3	6	0		0		7	33	4	19	2	13	2	11	0		0	
1:120–1:240	6	13	0		0		1	5	3	14	0		0		0		0	
1:240	10	21	1	2	0		0		2	10	0		0		0		0	
		% Neg.		% Neg.		% Neg.		% Neg.		% Neg.		% Neg.		% Neg.		% Neg.		% Neg.
Negative in all dilutions	10	21	30	63	39	81	0		5	24	12	75	4	22	6	33	13	72

* Aged 24 to 48 hours at room temperature.

The tendency of fresh malarial sera to show precipitation with antigen suspension is considerably greater than that of nonmalarial sera. In 6:1 and 12:1 ratios of serum: antigen suspension, 67 per cent of the malarial sera showed precipitation and 39 per cent and 35 per cent, respectively, of the nonmalarial sera showed precipitation. In the 24:1 ratios, 28 per cent of the malarial sera showed precipitation against 19 per cent of the nonmalarial sera. In the case of sera which have undergone 24 hours ageing at room temperature, 93 per cent, 87 per cent, and 81 per cent of the malarial sera showed precipitation in the 6:1, 12:1, and 24:1 ratios, respectively, and 62, 33, and 12 per cent of the nonmalarial sera showed precipitation in the same ratios, respectively. The greatest difference in the percentages of the precipitation reactions between fresh malarial and nonmalarial sera was found to be in the 12:1 ratio (67 per cent: 35 per cent); between aged malarial and nonmalarial sera was found to be in the 24:1 ratio (81 per cent: 12 per cent).

SUMMARY

It was desired to find the extent to which malarial sera would react with lipidal tissue extract antigens, when employing technical conditions favorable for eliciting nonsyphilitic reactions. Three antigens were employed, standard and sensitized Kahn antigens and specially prepared excessively sensitive antigen, with precipitation systems of low NaCl concentration and incubation at cold temperature.

1. It was found that sera of malarial origin showed a greater tendency toward precipitation with lipid antigens than sera of nonmalarial origin. Employing a precipitation system of 0.3 per cent NaCl concentration, instead of 0.85 per cent, with incubation at 1°–10°C., the ratio of precipitation reactions given by malarial and nonmalarial sera with standard antigen was 60 per cent: 32 percent; with sensitized antigen, the ratio was 47 per cent: 38 per cent; with excessively sentitive antigen, the ratio was 97 per cent: 59 per cent.

2. When malarial and nonmalarial sera were serially diluted with water and the dilutions tested with standard antigen suspension in a 0.3 per cent precipitation system, malarial sera showed a more marked tendency toward precipitation than nonmalarial sera. The age of the sera and the ratios used with antigen suspension affected the precipitation results. Thus, fresh malarial sera in a 12:1 ratio of serum: antigen suspension gave 67 per cent positive reactions against fresh nonmalarial sera which gave 39 per cent reactions. Malarial sera aged 24 to 72 hours and tested with antigen suspension in a 24:1 ratio gave 81 per cent positive reactions against similarly aged nonmalarial sera which gave 12 per cent reactions.

RESUMEN

Fué deseable encontrar a qué grado los sueros maláricos reaccionaban con antígenos de extracto de tejido lípido,—cuando se usaron condiciones técnicas favorables para despistar reacciones no sifilíticas. Fueron empleados tres antígenos, antígeno standard, antígeno sensibilizado de Kahn y antígeno extremadamente sensibilizado, especialmente preparado, con sistemas de precipitación de baja consentración del cloruro de sodio y una incubación a temperatura fría.

1.—Se encontró que los sueros de origen malárico mostraron una mayor tendencia a la precipitación con los antígenos lípidos que los sueros de origen no malárico. Cuando se empleoun sistema de precipitación con una concentración de NaCl de 0.3 por ciento, en vez de usar 0.85 por ciento, con incubación a una temperatura de 1° a 10° C, la proporción de las reacciones de precipitación dadas por los sueros maláricos y no maláricos con antígeno Standard fue de 60 por ciento: 32 por ciento; con antígeno sensibilizado la proporción fue 47 por ciento: 38 por ciento; con antígeno extremamente sensibilizado la proporción fue 97 por ciento: 59 por ciento.

2.—Cuando los sueros maláricos y no maláricos fueron diluídos en serie con agua y las diluciones probadas con una suspensión de antígeno standard en un sistema de precipitación de 0.3 por ciento, los sueros maláricos mostraron una tendencia más marcada a la precipitación que los sueros no maláricos. La edad de los sueros y las proporciones usadas con la suspensión de antígeno afectaron los resultados de la precipitación. Así pues los sueros maláricos frescos en proporción de 12:1 de suero:

suspensiónde antígeno dieron el 67 por ciento de reacciones positivas en comparación con sueros frescos no maláricos que dieron 39 por ciento de reacciones. Los sueros maláricos de edad de 24 a 72 horas y probados con suspensiones de antígeno en la proporción 24:1 dieron 81 por ciento de reacciones positivas en comparación con sueros no maláricos de la misma edad que dieron el 12 por ciento de reacciones.

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NATIONAL MALARIA SOCIETY

Meeting Conjointly With The Southern Medical Association

MINUTES, 1946

OFFICERS

Honorary President—Mr. J. A. LePrince, Memphis, Tennessee
President—Dr. Mark F. Boyd, Tallahassee, Florida
President Elect—Mr. Mark D. Hollis, Atlanta, Georgia
Vice President—Mr. J. A. Mulrennan, Jacksonville, Florida
Secretary-Treasurer—Dr. Martin D. Young, Columbia, S. C.

Tuesday, November 5, 1946

The National Malaria Society convened for its 29th annual session in the Hotel Everglades, Miami, Florida, at 2:00 p.m. with Dr. Mark F. Boyd presiding. The President appointed temporary nominating and auditing committees.

A program consisting of 12 papers, most of which were illustrated by lantern slides, was presented. The meeting adjourned at 5:10 p.m.

Wednesday, November 6, 1946

9:00 a.m.

The Society reconvened in the Hotel Everglades in a joint session with the American Society of Tropical Medicine with Dr. Mark F. Boyd and General J. S. Simmons, the Presidents of the respective societies, jointly presiding. Seven papers were presented, and 2 presented by title. The meeting adjourned at 11:40 a.m.

Thursday, November 7, 1946

9:00 a.m.

The Society reconvened with Mr. J. A. Mulrennan presiding. Nine papers were read and one presented by title. The paper by Dr. Justin Andrews was discussed by Captain Omar Brown, of the U. S. Navy, who had been invited to present the Navy's experience with malaria during the last war.

On behalf of the Society, Dr. L. L. Williams, Jr., presented a gift consisting of a six piece sterling silver tea service, to Dr. Mark F. Boyd in recognition of his service to the Society and of his contributions to the knowledge of malaria and its control.

After a brief intermission, the National Malaria Society held its business meeing with Dr. Boyd presiding. The minutes of the 1945 annual meeting, held in Cincinnati, were approved as published in Volume 4, No. 1 of the Society's Journal.

The Secretary-Treasurer reported as follows:

From the 1945 roster of 424 active members, one (Dr. Craig Barrow) has been lost by death, 10 by resignation, and 24 have been dropped because of delinquency in dues. One hundred thirteen members have been gained by election, making an active membership list of 504, representing a gain of 80 members; of these 408

are in good standing as of October 31, 1946. Of the 21 honorary members on the 1945 roster, 2 (Mr. Frederick L. Hoffman and Dr. M. J. Rosenau) have been lost by death, leaving 19 honorary members. The active and honorary membership totals 523.

The status of the treasury, as of the close of business on October 31, 1946, was:

Balance of November 10, 1945	$4,876.46	
Receipts from delinquent, current and advance dues, subscriptions, advertising, and interest on savings	2,821.76	
		$7,698.22
Expenditures before paying for the 2nd, 3rd, and 4th issues of the Journal—Volume 5		2,671.52
Balance		$5,026.70

Of the balance on hand October 31st, $4,812.10 is in the publication fund.

Assets estimated for 1946, including the above cash balance, total $5,481.70. Estimated liabilities are $2,093.80, which leaves the estimated net resources available at the end of the year to be $3,387.90.

There was a drop in revenue for the Journal due to the cancellation of 198 army subscriptions because of inactivation of malaria units overseas.

The Auditing Committee's report was presented by Mr. J. L. Porter who stated that the accounts of the Secretary-Treasurer had been found to be correct and he moved the acceptance of the above report, and also that honoraria be granted of $100 to the Secretary's stenographer and of $25 to the Editor's stenographer, respectively. This motion was carried.

Dr. Williams moved that "suitable resolutions be drawn upon the death of three members of the Society and copies of these be transmitted to the families of the deceased." The motion was approved, and the Secretary was instructed to draw up the resolutions.

Mr. F. L. Knowles gave the report of the Editorial Board. It was moved and carried that this report be accepted.

Reports were submitted from the other committees, as follows: Medical Research by Dr. V. H. Haas, Statistics by Dr. E. C. Faust, and Membership by Dr. Wendell Gingrich. In the absence of the committee members, the report of the Committee on Industrial Relations was read by the Secretary. Other reports received by the Secretary but not read were from the Committees on Entomology and Sanitary Engineering. These were accepted with thanks and were ordered to be submitted to the Editorial Board. It was moved by Dr. Williams that committee reports too long to be published be abstracted for possible publication. This motion was adopted.

Mr. J. L. Porter offered the suggestion that the Society consider broadening the scope of its activities to include the whole field of insect transmission of disease. No formal action was taken, but it was directed to the attention of the incoming officers.

Consideration was then given to the adoption of the new Constitution and By-Laws which had been proposed to the Society and presented at the last meeting. Each was read by sections. Five minor changes in the Constitution and three in the By-Laws, dealing mainly with clarification and better construction, were adopted by motion.

It was moved that the Resolutions Committee be thanked for its constructive work upon the Constitution and By-Laws. The motion was carried and the Secretary was instructed to transmit this action to the Committee.

Moving to new business, Dr. Boyd suggested that Dr. Williams speak of the plans of the State Department for the International Congress on Tropical Medicine and Malaria. Dr. L. L. Williams, Jr. indicated the approval of the Department of State for sponsoring and helping arrange for an International Congress on Tropical Medicine and Malaria; a budget for tentative arrangements has been approved. The best date appears to be between January and April of 1948. They are prepared to make meeting and space arrangements and to publish the proceedings. It is expected that representatives of various groups be called soon to discuss the whole program. These representatives are to give the information for the various societies. It was further brought out by Drs. Boyd and Williams that a membership and dues arrangement may be set up for interested workers.

The report of the Nominating Committee was present by Mr. Rector. In conformity with the newly adopted Constitution, the following nominations were presented:

President—M. D. Hollis
President Elect—E. Harold Hinman
Vice President—Wendell Gingrich
Director for one year—Justin M. Andrews
Director for two years—L. M. Clarkson
Director for three years—W. H. W. Komp

There being no further nominations from the floor, Dr. Williams moved that the nominations be closed and that the Secretary-Treasurer be instructed to cast the unanimous ballot of the Society for the nominees. The motion was carried.

It was moved that the Board of Directors "be instructed to consider the expanding interests of the Society." This motion was also carried.

The Secretary was directed to send appropriate thanks on the behalf of the Society to the Southern Medical Association, the Dade County Medical Society, the Hotel Everglades, and to Mr. Fred Stutz, for their interest and support in making the meetings a success.

There being no further business, the meeting adjourned *sine die* at 1:25 p.m.

MEMBERS WHOSE ADDRESSES ARE UNKNOWN

The Secretary needs the current addresses of the following members. Their last known addresses are shown. Anyone knowing where any of these can now be reached would do a favor by informing the Secretary's office.

Benjamin, Major MacB.
Mitchell Convalescent Hospital
Camp Lockett, California

Bobb, Lt. (jg) Marvin L., USNR
Piedmont Research Laboratory
Charlottesville, Virginia

Bruno, Asst. Engr. (R) Ralph D.
Box 190, Elizabeth City, N. C.

Burlingame, Captain Paul L.
AAF Regional Hospital, SAAAB
Santa Ana, California

Butler, Lt. Philip A.
10th Malaria Survey Unit
APO 600, New York City

Connell, Lt. Walter A.
22nd Malaria Survey Unit
APO 709, c/o Postmaster
San Francisco, California

Cranford, Captain C. A.
1st Malaria Control Unit
APO 37, c/o Postmaster
San Francisco, California

Dicke, Lt. (jg) Robert J.
Medical Office, NSNATB
Fort Pierce, Florida

Durham , Major Gen. George C.
2231 California St., N.W.
Washington 8, D. C.

Grimsley, Assoc. PH Engr. Joseph T.
c/o City Health Department
Houston, Texas

Hemmings, Lt. R. J., USNR
Malaria Epidemic Control Unit 16
2nd Med. Batt., 2nd Marine Div.
F.P.O., San Francisco, Calif.

Hodgden, Lt. Burton B.
Station Hospital
Camp Gordon Johnston, Fla.

Kittrell, Captain W. H.
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603 B.M.A. Building
Kansas City 8, Missouri

Minter, Major David R.
Rochdale, Mississippi

Quenelle, Captain Owen G.
AG & SF Redistribution Station
Asheville, North Carolina

Rice, Colonel Earle M.
2 Greenhill Street
Charleston 21, S. Carolina

Smith, Captain Millard E.
Station Hospital
568th AAFBU (4th OTU), GAAF
Greenwood, Mississippi

Welt, Dr. Louis G.
7 May Street
Hartford, Conn.

Whorton, Dr. Carl M.
Valley Forge Gen. Hospital
Joliet, Illinois

Wilson, Dr. Henry E., Jr.
University Hospital
Columbus, Ohio

Young, Lt. Roy T., Jr. USNR
Area 2, Building 221
Camp Lejeune, North Carolina

CONSTITUTION AND BY-LAWS
NATIONAL MALARIA SOCIETY

(Revised and adopted Nov. 7, 1946.)

CONSTITUTION

1. *Name.* The name of this organization shall be "The National Malaria Society."

2. *Purpose and Functions.* The object and purpose of the Society shall be to advance knowledge regarding the cause, prevalence, epidemiology, treatment, prevention and control of malaria through integration of activities of the various specialized fields of endeavor and through stimulation of scientific and practical interest among organizations and individuals in the prompt and effective application of treatment and control methods.

3. *Membership.* Any person who has shown scientific or practical interest in malaria and the control thereof may be proposed for membership in the Society and may be elected to membership as provided by the by-laws of the Society.

4. *Officers.* The officers of the Society shall be a President, a President-Elect, a Vice President, a Secretary-Treasurer, a Business Manager for the Journal, and three Directors.

These officers, with the latest living Past-President continuing to be a member of the Society, shall constitute the Board of Directors in which the government of the Society shall be vested.

The Secretary-Treasurer and the Business Manager for the Journal shall be appointed by the Board of Directors, and at the discretion of the Board the same person may hold both offices.

The terms of office of the President, President-Elect, and Vice President shall be for one year.

The terms of office of the Secretary-Treasurer and the Business Manager for the Journal shall be for three years.

The terms of Directors shall be for a period of three years, except that the terms of the Directors first elected under this constitution shall be for one, two and three years, respectively.

Terms of office shall begin at the close of the annual meeting at which the officers are elected and shall continue until successors are qualified.

A vacancy in the office of the President shall be filled by the Vice President.

A vacancy in the office of the Vice President shall be filled by the Senior Director.

5. *Nomination and Election of Officers.* At the annual meeting, the President shall request that suggestions for nomination of officers to be elected in the next year be sent to the Secretary, who shall turn the names over to a nominating committee of three members, to be appointed by the President not later than July 1.

Not later than September 1 of each year, the nominating committee shall report to the Board of Directors at least one nominee for each office, the terms of which expire during the year, and for such other vacancies as may exist. The name of

any member shall be included if ten members have so requested. Any one name shall appear as a candidate for only one office.

The Board shall cause a ballot to be sent to each member.

Ballots shall be returned to the Board at least ten days prior to the date of the annual meeting of the Society at which time they will be counted.

At the annual meeting the President shall cause the ballots to be canvassed by the committee appointed for that purpose.

The results of the election shall be announced at the first session of the annual meeting.

6. *Eligibility to Office.* Any member in good standing may be elected to any one of the offices of the Society.

7. *Meetings.* The National Malaria Society shall hold one regular meeting each year at the time and place of the annual meeting of the Southern Medical Association, if held, or otherwise at a time and place designated by the Board of Directors.

8. *Representative to the Council of the American Association for the Advancement of Science.* The President shall appoint biannually from among the members of the Society who are concurrently Fellows of the American Association for the Advancement of Science, a representative of the Society to the Council of the A.A.A.S.

9. *Amendments.* By-Laws may be amended at any annual meeting by a majority vote of members present and voting.

Proposed amendments to the Constitution shall be submitted to the Board with the endorsement of at least five members. The Board shall prepare a digest of the arguments pro and con for the proposal and submit it to the Society at the annual meeting. Those present shall vote on the proposal by ballot. If two-thirds of those present and voting are favorable to the proposal it shall be submitted to the consideration of all members by mail ballot before September 1. The mail ballots shall be canvassed at the following annual meeting. If the proposal is favored by two-thirds of those balloting, the amendment shall go into immediate effect.

10. *Editorial Board.* The President is hereby authorized to appoint, with the approval of the Board of Directors, an Editorial Board of three members in good standing, the members of the first of which shall hold office for terms of one, two and three years, respectively. Thereafter, one member shall be appointed annually for a three years' term. The Secretary shall be ex-officio a fourth member but shall not serve as editor.

There is hereby continued the *Journal of the National Malaria Society*, the editorial direction of which shall be managed by the Editorial Board as provided in the By-Laws.

The Editorial Board shall elect one of its member as editor of the Journal.

BY-LAWS

1. *President.* The President shall have general supervision of the affairs of the Society. He or his designate shall preside at meetings of the Society and of the Board of Directors. He shall be ex-officio member of committees. He shall deliver an address at the annual meeting.

2. *Vice President.* The Vice President shall preside at meetings in the absence of the President, and shall discharge the duties of the President in case of a vacancy in that office.

3. *Directors.* The Board of Directors shall manage the affairs of the Society. Specifically, the functions of the Board shall include, but shall not be limited to, the following:

a. Take measures to advance the interests and aims of the Society; generally direct its business; review all applications for membership; coordinate the work of the various committees, and, at its discretion, request committees for reports on particular phases of general assignments; prepare a program for the annual meeting; appoint a Secretary-Treasurer of the Society and Business Manager for the Journal; have the power to order all ordinary and current expenditure of funds; and perform other duties.

b. The President, subject to the approval of the Board of Directors, is authorized to appoint committees of its members, or of the Society, and to delegate to them such powers as it may deem fit; provided, however, that any action of the committee unfavorable to any member or applicant for membership, shall be reported to the whole Board for action.

c. Establish an annual budget for the publication of the Journal.

4. *Secretary-Treasurer.* The Secretary-Treasurer, as an aide to the Board of Directors, shall assist in the management of the Society's business affairs; shall collect the dues and receipt therefor; shall keep a bank account in the name of the Society in which current funds are deposited; shall disburse funds only on the approval of the Board, and shall account for moneys received and disbursed on account of the Society. He shall conduct, under the direction of the Board, the routine correspondence of the Society. He shall record the minutes of the meeting.

On payment of the annual dues, two ($2.00) dollars thereof shall be set aside by the Treasurer as a subscription to the Society's Journal during the corresponding year for the member so paying. The issues of the current volume of the Journal shall not be distributed to members prior to the payment of their dues for the current year, nor to members in arrears or delinquent. Members who apply for re-instatement by payment of delinquent dues shall be required to pay one ($1.00) dollar for each year of their delinquency, but they shall not be furnished copies of the Society's Journal for the period of their delinquency. They may secure available back issues of the Journal at pro-rata cost, if available.

He shall keep in a special publication fund the two ($2.00) dollars subscription to the Journal annually paid by members of the Society and all other revenues derived from subscriptions paid by non-members, advertising and the sale of extra copies of back issues. This special fund may be drawn upon only to pay the publication and distribution costs of the Journal.

The Secretary-Treasurer shall be bonded. The Board of Directors shall determine amount of the bond and the cost thereof shall be paid by the Society.

5. *Business Manager for the Journal.* The Business Manager for the Journal shall serve as an aide to the Editorial Board and shall work in close cooperation with the Secretary-Treasurer of the Society.

6. *Membership and Dues.* Nomination for membership shall be submitted in writing to the Board, with endorsement by two members.

Unanimous approval by the Board of Directors shall constitute election to membership.

The dues of members shall be three ($3.00) dollars per annum and shall become due and payable on the 1st of January each year. When any member shall be in arrears for two years, his membership shall automatically terminate.

7. *Program. Scientific:* The Board of Directors shall arrange a program and may invite contributions. Members shall have the privilege of offering papers to be presented at the annual meeting.

Business: In the conduct of the business sessions, *Robert's Rules of Order* shall be the guide to parliamentary procedure.

8. *Editorial Board Publication of papers and reports.* The Editorial Board shall review the manuscripts of all papers, including reports of scientific committees with a view to their publication in the Journal.

The Editorial Board shall be empowered to contract or otherwise arrange for the printing of the Journal, subject to the approval of the Board of Directors.